Post- mortem investigation of widespread cortical changes in patients with epilepsy and hippocampal sclerosis

THE INSTITUTE OF NEUROLOGY

SUBMITTED IN PART FULFILMENT FOR THE MSc IN CLINICAL NEUROSCIENCE, UNIVERSITY OF LONDON, 2008

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2007/08

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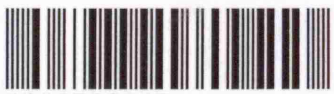

ACKNOWLEDGEMENTS

I would like to give warm thanks to my supervisor Dr. Maria Thom and Mrs. Lillian Martinian who have helped me throughout the duration of this project. A special thank you to Professor Sanjay Sisodiya for his useful suggestions.

All my love and thanks to my family who are always there for me.

STATEMENT OF CONTRIBUTIONS
Study Design: Maria Thom, Francine Blanc
Methods: Maria Thom, Lillian Martinian
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ABBREVIATIONS

The following abbreviations have been used throughout the text:

AEDs- Anti-epileptic drugs

CHS- Classical hippocampal sclerosis

DTI- Diffusion Tensor Imaging

DWI- Diffusion Weighted Imaging

EEG- Electroencephalogram

EFG- End folium gliosis

GFAP- Glial fibrillary acidic protein

HS- Hippocampal sclerosis

ILAE- International League Against Epilepsy

MRI- Magnetic Resonance Imaging

MTS- Mesial temporal sclerosis

MTLE- Mesial temporal lobe epilepsy

PWI-Perfusion Weighted Imaging

TLE- Temporal lobe epilepsy

TLS- Temporal lobe sclerosis

VBM- Voxel-based Morphometry

ABSTRACT

Hippocampal sclerosis is a common pathological feature in temporal lobe epilepsy patients and may actually be an indicator of more widespread neocortical changes, which may be asymmetrical. There is some suggestion from neuroimaging research that these widespread changes may involve hippocampal projection sites such as the cingulate gyrus and entorhinal cortex. The aim of this study was to investigate, using quantitative post-mortem techniques, whether or not patients with epilepsy and hippocampal sclerosis have more widespread neocortical changes. Six hippocampal sclerosis cases were compared with four neurologically normal controls, using field fraction analysis for quantitative assessment, in order to measure the intensity of GFAP staining present in selected cortical and white matter cerebral regions. No overall differences in gliosis were found between the patients and the controls, however hippocampal projection regions were shown to be more gliotic than the same regions in the controls, as well as more gliotic in the hemisphere ipsilateral to the sclerosis. These preliminary findings suggest that there is more widespread damage occurring in both the grey and white matter of patients with hippocampal sclerosis and this is important when considering possible reasons as to why some patients who undergo resective surgery may continue to experience seizures post-operatively.

1. INTRODUCTION

1.1. The Problem

Mesial temporal lobe epilepsy is the most common of the symptomatic epilepsy syndromes (Engel, 2001) and hippocampal sclerosis is the most strongly associated pathological abnormality, found in the majority of patients. Unfortunately, this epilepsy syndrome is also the most common drug refractory type and many patients fail to successfully respond to treatment with anti-epileptic medication. Nevertheless, such patients may be effectively relieved of their seizures via surgical resection the mesial temporal lobe structures, which are believed to underlie their seizure genesis. Still, this is not a perfect treatment as a subset of patients may continue to experience seizures post-surgery or enjoy a seizure free period for months-years but still suffer from repetitive seizures after this time. McIntosh et al., (2006) showed that patients experiencing post-operative seizures only had a 26.5% chance of remaining seizure free after 2 years. In fact, 20-30% of patients still experience seizures after surgery (Weiser et al., 2003). This issue is discussed more extensively in Chapter 2.3.2.

Imaging studies conducted by Cormack et al., (2005) and Lin et al., (2007) have suggested that patients with mesial temporal lobe epilepsy may actually have more widespread neocortical change, which extends beyond mesial temporal lobe structures. Establishing whether or not this is true may be important, especially in the light of causes for unsuccessful surgical resections in patients.

1.2. Purpose and Significance of this Study

This study aims to investigate, via post-quantitative immunohistochemistry, whether or not hippocampal sclerosis is associated with more widespread neocortical change in mesial temporal lobe epilepsy.

For patients who continue to have seizures after surgical resection for hippocampal sclerosis and for those who experience recurrent seizures several months post-surgery there are several explanations including:

1. The location of the tissue thought to be responsible for seizure genesis was inaccurate or was not fully removed
2. Experience of long-term repetitive complex partial seizures, which results in extra-temporal areas becoming independently epileptogenic (Lin et al., 2007)
3. There is more widespread damage occurring including extra-temporal damage, contralateral temporal damage or, as recent neuroimaging studies are beginning to suggest, widespread neocortical damage, which may be linked to point 2.

If the latter is the case, seizures may be being evoked by non-mesial temporal lobe structures, thus by the time patients have undergone surgery, other intact areas may be responsible for the generation of seizures. To date, no quantitative post-mortem study has been conducted to investigate whether or not further structural neocortical changes occur in patients with hippocampal sclerosis and mesial temporal lobe epilepsy. Any existing studies employ neuroimaging, which do not allow for investigation of microscopic changes in brain tissue.

This is an important area of research as it may assist in primarily explaining, and in the future identifying, why some patients fail to respond to surgery and secondarily, why patients with this epilepsy may experience cognitive and psychiatric disturbances. Furthermore, seizures have a strong impact on patients' quality of life, thus full prevention is essential.

1.3 Outline Of Dissertation

The following chapter (Chapter 2) will explore and evaluate the literature in this area, leading to the research hypothesis. Following on from this, Chapter 3 will detail the

methodology of this research and Chapter 4 the research findings, with details of statistical methods used. Chapter 5 will evaluate the findings and their possible implications, provide suggestions for further study as well as highlight problems encountered whilst doing the study and limitations of the research.

2. LITERATURE REVIEW

2.1. Epilepsy: Epidemiology and Aetiology

Epilepsy describes a condition in which sufferers are prone to recurrent epileptic seizures. It should not be considered as a disease itself but rather as a group of syndromes indicative of abnormal neuronal activity. According to Sander (2007), the disorder is one of the most serious neurological conditions. Despite the severity of the syndrome incidence rates are difficult to compute due to its variability as well as differences in diagnosis and inclusion criterion in research studies (Sander & Shorvon, 1996). Some patients may even be unaware that they have epileptic seizures and thus this as well as the previous aforementioned issues, result in difficulty when trying to compute epidemiology rates.

The International League Against Epilepsy (ILAE) states that in Europe there is an age adjusted annual incidence of approximately 40 to 70 per 100,000 persons (ILAE, 2003). In developing countries the rates are often higher (Sander & Shorvon, 1996), the exact reasons for which are unknown however it has been speculated that lower socioeconomic status and poverty as well as restricted access to medication may be related to the higher incidence rates, amongst several other mediating factors (Jallon, 1997).

The spectrum of epilepsy may be separated into different seizure types, different electrophysiology patterns as well as different anatomical sites and pathological causes of seizures. Thus the aetiology of epilepsy is several fold and is unfortunately, still poorly understood. The various different types of seizures and subsequently different types of epilepsy further complicate matters.

Based on their electroclinical features, seizures may be separated into being generalised, or partial epilepsies. Furthermore, if they are the result of a known cause they can be

labelled as symptomatic, or idiopathic if the cause is unknown but may have a genetic basis (ILAE, 2003) as well as cryptogenic if the cause is unknown and believed to be due to some other non-genetic factor. The ILAE (2003) report a large range of aetiologies resulting in epilepsy including metabolic disorders, genetic lesions, head injuries, cerebral infarcts, brain tumours and Ammon's horn sclerosis, the latter being the focus of this research project.

This project will be focusing on mesial temporal lobe epilepsy (MTLE), which is a symptomatic syndrome that commonly yields partial seizures. It is largely associated with Ammon's horn sclerosis, now more commonly known as hippocampal sclerosis (HS), which is said to account for up to 70% of all cases of complex partial seizures (Engel, 1987). In order to gauge a better understanding of the nature of HS it is necessary to understand the anatomy of the hippocampus.

2.2. Anatomy of the Hippocampus

The hippocampus is part of the hippocampal formation, which also consists of the dentate gyrus and areas of the parahippocampal gyrus such as the subiculum and entorhinal cortex (Crossman and Neary, 2005). Often the hippocampal formation is divided into three regions: the dentate gyrus, the subicular complex and Ammon's horn, which is also known as the hippocampus proper (Fisher et al., 1998). The hippocampi are a pair of curved structures, named so because of their resemblance to a seahorse (*Hippocampus*). They sit subcortically in the brain, located lateral to the thalamus and medial to the temporal horn of the ventricles. Tissue sections of the hippocampus show a clear, layered arrangement of neuronal cell bodies and fibre tracts (Cooper and Lowenstein, 2003). Hippocampal pyramidal cells (in Ammon's horn) are located in one layer within different regions known as CA1, CA2, CA3 and CA4. The cells of these

regions differ from each other both in appearance and connections and as will be highlighted later, different regions have been implicated in HS.

Connections of the hippocampus are both intrinsic, i.e. between layers, as well as extrinsic. Projections are sent to and received from various brain areas. Area CA1, for example, is associated with receipt of projections from the perirhinal and postrhinal cortices as well as the basal nucleus of the amygdala (which also receives projections from CA1) and in turn also projects to the medial frontal lobe (Amaral and Lavenex, 2007). Therefore it is possible that seizures generated in the hippocampus may spread to connected areas and so over a period of time extra-hippocampal structures may too become damaged or atrophic as is suggested by several imaging studies that will be explored later in this chapter.

2.3. Temporal Lobe Epilepsy and Hippocampal Sclerosis

Temporal lobe epilepsy (TLE) is an example of a disorder that induces symptomatic seizures i.e. the disorder arises due to localised structural abnormalities. This is in contrast to other epilepsy types such as idiopathic epilepsy, where the exact cause is unknown but genetics are believed to play a part. Typically epileptogenesis is observed in the mesial temporal lobe structures with accompanying mesial temporal sclerosis (MTS) pathology (Engel, 2001). MTS is a term used to describe neuronal loss in the hippocampus as well as in the amygdala and neighbouring entorhinal cortex (Chang & Lowenstein, 2003; Falconer & Taylor, 1968).

According to Engel, Williamson and Wieser (1997), MTLE with HS has a distinguishable clinical presentation. Typically, patients have been found to have a history of childhood febrile seizures in the first five years of life or a preceding head injury as well as a higher probability of family history, suggesting there may be some genetic influence.

Spontaneous afebrile seizures coupled with complex partial seizures may also occur

during childhood, which at first respond well to medication resulting in the individual being seizure free for many years. However, intractable forms may result in recurrence of seizures during early adulthood and these tend not to respond to medication administered (Engel, 2001).

Both simple and complex partial seizures may be experienced in MTLE, with the former being distinguished from the latter by level of consciousness (Duncan, 2007). Complex partial seizures may be recognised by a preceding aura occurring prior to the seizure, which may include feelings of fear, paramnesia and various autonomic symptoms, followed by motor arrest with motionless staring and oro-alimentary automatisms such as chewing and lip-smacking as well as motor automatisms (Duncan, 2007; Engel, 2001). Post-ictally amnesia and confusion often occur, the former is of no surprise as the seizure site of origin is the temporal lobes, which are known to have strong correlations with memory.

2.3.1. Pathology of Hippocampal Sclerosis

HS is the most common neuropathological finding in MTLE lobectomies. From a historical perspective, this particular discovery was first described by Sommer (1880) (cited from Walker, Chan and Thom, 2007) and histologically described by Bratz in 1899 (cited from Fisher, Sperber and Moshé, 1998). Sommer highlighted that predominately, in region CA1, there was loss of pyramidal neurons as well as gliosis in the brains of chronic epilepsy patients. It was subsequently proposed that these markings were the cause of epilepsy, however since then it has been argued that the lesions may actually be an artefact of the seizures rather than the cause. It does however appear more likely that seizures originate in this area due to several lines of evidence; for example it is commonly known that patients who undergo surgical resections of the sclerosed hippocampus experience a remission of their seizures and

electroencephalogram (EEG) recordings from the area have suggested that seizure onset is initiated here (Walker et al., 2007; Falconer, 1974).

HS results in an overall reduction in the volume of the hippocampus (Thom, 2007), which can be observed using neuroimaging techniques such as magnetic resonance imaging (Fig.1.). Microscopic analysis, via immunohistochemical staining for example, aids in highlighting more in-depth cellular changes (see Fig. 2). Glial fibrillary acidic protein (GFAP) immunohistochemical staining is useful for the measurement of astrogliosis in sclerotic hippocampi. GFAP stains in HS shows widespread gliosis in CA1 subfields as well as in the granule cell layer (Thom, 2007). The more intense GFAP staining in sclerosed tissue is indicative of reactive astrocytosis, which is indicative of brain injury (Kraig et al., 1991). Cresyl violet stains can also be useful to observe neuronal and glial loss (see Fig. 2.).

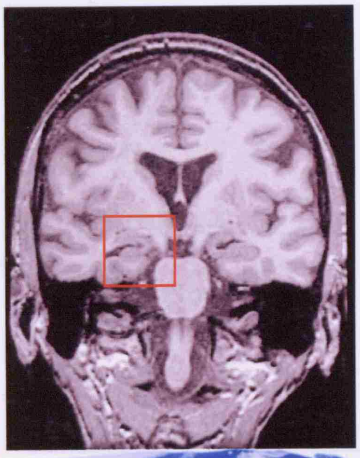

Figure 1. A T1-weighted scan showing hippocampal atrophy on the right in a patient with mesial temporal sclerosis. Adapted from: Cascino (2001)

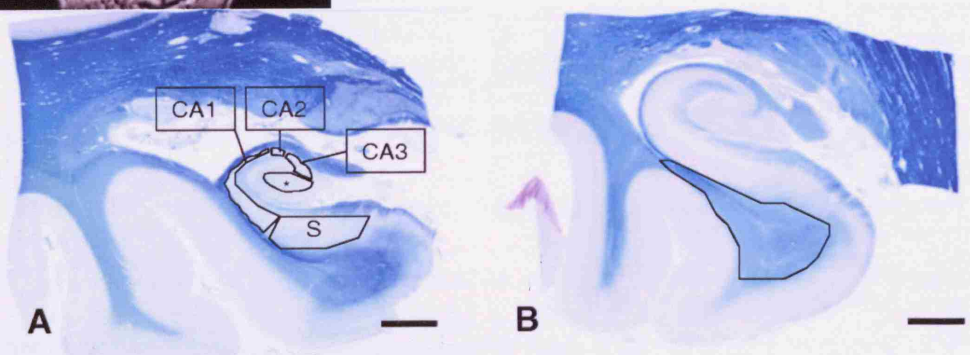

Figure 2. Quantification of the hippocampal subfields (A), comparing hippocampal sclerosis pathology (A) against no hippocampal sclerosis pathology (B). Sections stained with Cresyl violet. Adapted from: Thom et al., 2005.

The ILAE (2004) commission report of mesial temporal lobe epilepsy with hippocampal sclerosis highlights that synaptic reorganisation and granule cell dispersion are additional pathological markers of HS, which may not be seen in all patients but a subset of cases. Furthermore they highlight the presence of extrahippocampal changes, which occur alongside HS however such findings have yet to be sufficiently analysed via pathological study (Weiser, 2004).

HS is not however confined to the hippocampus alone, with the pathology known to extend to the temporal neocortex. In a volumetry study using patients with intractable TLE, Moran et al., (2001) found widespread temporal lobe volume loss within all temporal lobe structures, with the exception of the amygdala. The latter is a peculiar find, as the amygdala has been known to be atrophic in intractable TLE patients. The authors hypothesise that the discrepancy may be due to the difficulty in identifying reliable boundaries for the amygdala in their measurement techniques. Nevertheless, volume reductions were identified in the lateral occipitotemporal/inferior temporal and middle temporal gyri, the superior temporal gyrus as well as the parahippocampal and medial occipitotemporal gyri, in addition to the hippocampus. Thom et al., (2001) identified several pathological correlates in a series of surgically treated patients with HS, who had temporal lobe sclerosis (TLS), i.e. extra-hippocampal damage. The group found TLS to be characterised by a reduction of neurones from cortical layers II and III, laminar gliosis as well as architectural abnormalities of layer II suggestive of developmental cortical malformations. There is therefore a possibility that such features may be present in other areas of the neocortex, though this is yet to be explored.

It has been well established that the presence of HS or MTS is a favourable prognostic outcome for patients undergoing surgery who have drug refractory temporal lobe epilepsy (Berkovic et al., 1995).

2.3.2. Surgery for Temporal Lobe Epilepsy and Outcome Studies

Standard treatment for the majority of epilepsies involves the patient taking anti-epileptic medication. This method is generally successful for many patients however there is still a fairly large subset of patients who do not respond to any anti-epileptic drugs (AEDs). This is very apparent in MTLE as several patients have drug refractory forms. In such cases, epilepsy surgery is recommended in which the area thought to be responsible for seizure genesis, which is usually the hippocampus, is resected. This only occurs after rigorous investigations such as EEG, MRI and telemetry are carried out. Conducting these investigations aims to preserve the unaffected cortex (Rosenow & Lüders, 2001) as well as reduce post-surgical side effects.

The most common procedures are anterior temporal lobectomies or selective amygdalohippocampectomies. In anterior temporal lobectomies there is complete removal of the anterior temporal lobe, whereas selective amygdalohippocampectomy aims to spare the lateral neocortex (Renowden et al., 1995). The literature at present shows favourable and unfavourable outcomes with both methods, with some studies citing anterior temporal lobectomies as having more favourable outcomes in terms of seizure freedom (Özkara et al., 2008) but others highlighting selective amydalohippocampectomies as having more favourable neuropsychological outcomes (Morino et al., 2006). If HS is present, the ILAE (2004) recommend resection of the hippocampal formation and the anterior parahippocampal gyrus.

In a well described, randomised control study comparing the effectiveness of surgery in temporal lobe epilepsy patients to treatment with AEDs, Wiebe et al., (2001) found that 58% of patients with temporal lobe epilepsy placed in the surgical group (n=40) were seizure free at one year's follow up in comparison to just 5% in the medical group (n=40). At this time, there had been a lack of randomised control trials in this area of

research and Wiebe et al.,'s findings demonstrated a clear superiority of surgery over medical treatment in temporal lobe epilepsy patients.

Lowe et al., (2004) investigated more specifically, seizure freedom (amongst other variables) amongst temporal lobe epilepsy patients with pathologically proven hippocampal sclerosis after surgery. This was a retrospective study, thus patients were recruited if they had had an anterior temporal lobectomy between 1993 and 1999 at the Royal Melbourne Hospital in Australia. Of the 50 suitable patients identified, 42 provided complete responses to the surveys issued. Of this cohort, 12 months after the initial surgery 82% were seizure free and 24 months after 76% were seizure free. Long-term outcome measures 63 months after surgery showed 64% remained completely seizure free. Unfortunately, there was no control group for comparison and a lack of presurgical information concerning patients' seizure rates per month or per annum. It is presumed that they were severe enough to undergo surgery however and were presumably drug refractory however the authors did fail to state this in the research paper. The results do show favourable outcomes for many of the patients however the authors highlighted that patients were at risk from seizure recurrence beyond 24 months after their surgery. The sample size of this group was also fairly small, limiting ability to draw definite conclusions.

A larger retrospective study conducted by McIntosh et al., (2004), used 325 patients who had undergone anterior temporal lobectomy between the years 1978 and 1998 and found that the likelihood of the entire sample remaining seizure free after 1 year and 2 years postoperatively was 61% and 55% respectively. Patients identified with HS did however have a significantly higher probability of being seizure free in comparison to those with absent pathology. Additionally in this group (n=116), 56 who remained seizure free after 2 years had discontinued AED medication. Still, there was a remainder (24%) who experienced late recurrent seizures after 10 years, postoperatively. It is not

detailed how many years the HS group remained seizure free for until initial seizure recurrence however. The authors state that their findings agree with those of Yoon et al's (2003), who found that 38% of those with MTS experienced recurrent seizures after being seizure free for just one year. On the other hand, McIntosh et al's group specified hippocampal sclerosis pathology and neglect to state the number of patients who after 1 year were not seizure free in this particular group. Yoon et al (2003) investigated MTS, which has a slightly more widespread pathology than just HS alone thus the extent to which the two groups' findings parallel each other may be questionable.

A common feature seen in these studies is the fact that surgical resections in the mesial temporal area for patients with drug refractory temporal lobe epilepsy are not always successful. There still remains a fairly large number of patients who immediately after surgery still experience recurrent seizures. Furthermore, the percentage of patients remaining seizure free in long-term follow-up studies (limited as they are) appears to decrease over the years.

There is an apparent lack of research, which adequately explores causes of failed surgery in patients with MTS/HS with MTLE. Hennessy et al., (2000) conducted a retrospective clinical study that employed different patient groups who had undergone temporal resections for temporal lobe epilepsy. The study included patients with MTS, dysembryoplastic neuroepithelial tumours and those with seizures who had non-specific pathology. 44 patients were reassessed who had recurrent or persistent postoperative seizures, amongst these 20 had MTS. Electroclinical study indicated that the majority did not have recurrent seizures, which arose from the residual hippocampus but actually appeared to be of neocortical origin. Neuroimaging with high resolution MRI however failed to detect gross or subtle dual pathology in the MTS patients. Although

neuroimaging is an extremely powerful tool, it cannot show detail that is visible only at the microscopic level. Considering the authors' postulations that the seizures could be of neocortical origin supports the hypothesis that MTS may be a pathological correlate that indicates involvement of extra-temporal pathology. On the other hand different mitigating factors, such as incomplete resections, should not be ignored as potential influencers of recurrent postoperative seizures.

However, in an early quantitative MRI study, Sisodiya et al., (1997) showed that patients with HS who had undergone hippocampal resection but still experienced postoperative seizures had additional extrahippocampal and cerebral structural abnormalities, including cerebral dysgenesis, suggestive of a dual pathology. In their chosen cohort, the researchers believe inadequate resection does not explain fully the results found due to their inclusion criteria requiring patients to have undergone hippocampal excision as far back as the anterior half of the midbrain, which Nayel et al., (1991) showed to be adequate in order to achieve seizure freedom.

The aforementioned research by Hennessy et al., (2000) and Sisodiya et al., (1997) not only lends support to this paper's research hypothesis but highlights the importance of employing more rigorous techniques when selecting patients for surgical resection. Now, with the use of techniques such as diffusion and perfusion weighted imaging (DWI and PWI respectively) neuroimaging tools have become extremely sophisticated. In the future it may become possible to reliably image the aforementioned dual pathology that may occur in some patients with MTLE and MTS or HS, which will greatly assist with choosing candidates for surgery with more accuracy.

2.4. Widespread Neocortical Changes with Hippocampal Sclerosis

Fairly recent evidence from neuroimaging studies e.g. Bonilha et al., 2006; Lin et al., 2007 has suggested that hippocampal sclerosis may serve as an indicator of more widespread neocortical changes.

Cormack et al., (2005), using magnetic resonance imaging and voxel based morphometry (VBM), compared grey matter density changes in hippocampal projection sites in a group of children (n=30) who had mesial temporal lobe epilepsy with a control group of neurologically normal children (n=22). The researchers found grey matter reductions in the hippocampus, thalamus, parahippocampal gyrus, lateral temporal lobes, posterior cingulate and retrosplenial cortex. Additionally, a neuropsychology assessment found a high incidence of developmental and behavioural problems, including several DSM-IV diagnoses. The latter could be an additional indication of other affected brain regions away from the sclerotic area. In support of this finding, Oyegbile et al., (2004) found that patients with temporal lobe epilepsy performed significantly poorly in comparison with controls on neuropsychological tests of memory, intelligence, language, executive function and motor speed. The patients also had less educational attainment, which may be linked with being unable to attend school consistently due to the severity of their epilepsy.

Furthermore in Cormack et al's cohort, those with right MTS had notably lower IQ scores in both performance and verbal measures in comparison to the control group. Children with left MTS also had lower IQ scores but to a lesser extent than those with right MTS. This could be due to inconsistent attendance at school, as highlighted by Oyegbile et al's study (2004) or perhaps due more widespread incorporation of the temporal lobes and the frontal lobes at a pathogenic level, which affects their task

performance. Unfortunately details of the IQ tests were not provided, so it is difficult to ascertain which brain regions would be involved during testing.

The authors also highlight that that widespread reductions in grey matter density in frontal cortices were not observed as in previous studies conducted by Keller et al., (2002). Research by Keller et al., (2002b) was however conducted on adults with temporal lobe epilepsy, thus there is a possibility that the damage becomes more widespread with a longer disease duration. Moreover, Cormack's group failed to detail how many seizures experienced per month in their clinical description of the sample, thus it is unknown whether or not there is any correlation between seizure duration and frequency with the presence or extent of more widespread pathology.

What is apparent from this research is that extra- mesial temporal lobe pathology may occur as early as childhood. This could be suggestive of interrupted cortical development as well as early disruptions to hippocampal projection sites due to HS.

Keller et al's (2002b) study also employed VBM, investigating grey matter abnormalities in surgical candidates who had epilepsy of temporal lobe origin, lateralised to either the right or left hemisphere. Details of where and how they recruited participants are missing in the paper and like Cormack et al's research they fail to investigate relationships between seizure frequency and AED treatment, which may influence brain atrophy. Nevertheless, they found reduced grey matter in several brain areas, including the medial frontal lobes in left seizure onset patients and the left cerebellum in right seizure onset patients. These aforementioned findings were also correlated with duration of epilepsy and the age of onset, though as with all cross-sectional studies, cause and effect are difficult to disentangle.

A diffusion tensor imaging study conducted by Thivard et al, (2005) established that diffusion abnormalities in 35 patients with mesial temporal lobe epilepsy with

hippocampal sclerosis extended to the contralateral temporal lobe, the temporal pole as well as extratemporal regions such as parietal regions, the corpus callosum and cingulum. This again suggests that MTLE may not be as focal as initially believed.

When coming to compare results from this area of research, caution must be taken due to the various different techniques and sample populations used. Some studies have chosen retrospective samples, where the patients have already undergone surgery for their epilepsy (Lin et al.,2007), whereas others are undergoing assessment and eligibility for epilepsy surgery (Thivard et al., 2005; Bonilha et al., 2007). There are large variations in neuroimaging techniques, control groups and outcome measures.

Although neuroimaging studies are beneficial for highlighting extra-hippocampal changes, it is difficult to calculate exactly how much atrophy or gliosis is present and thus the exact pathological correlates remain unknown. Conducting post-mortem examinations on individuals with known hippocampal sclerosis is therefore beneficial for increasing accuracy in this area of research as well as conducting a closer examination of possible affected neocortical areas.

2.5. Aims and Hypothesis of Investigation

This research project will investigate, via post-mortem study, whether or not temporal lobe epilepsy patients with HS also have widespread cortical atrophy as has been suggested by the aforementioned neuroimaging studies. We will also investigate the presence of gliosis in the white matter to see if any changes also occur here. Based on the evidence from imaging studies, it is predicted that there will be a difference in cortical and white matter damage between MTLE patients and controls.

3. METHODS

3.1. Case and Tissue Selection

Post-mortem cases were selected from the archives of the Division of Neuropathology at the National Hospital for Neurology and Neurosurgery, Queen Square; London. The local ethics committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology has given approval for neuropathological epilepsy studies and era appropriate consent was obtained from patients' next of kin.

Six epilepsy cases (age range 31-84; mean age 54) were selected with histologically confirmed HS along with four controls (age range 36-78; mean age 57) without significant neurological disease or epilepsy (see Table 1. for patient and control demographics as well as neuropathological details of the cases). The HS was either unilateral (n=3) or bilateral (n=3). (see Table 2 for more clinical and neuropathological details). In the selection of the epilepsy cases, included were two cases (45-02 and 55-95) in which there was macroscopic impression of ipsilateral cortical atrophy in addition to HS (see Figure 3). We excluded any epilepsy cases with confounding secondary cortical pathology e.g. stroke, previous head injuries i.e. contusions and significant neurodegenerative disease. The controls were selected from the same era so that fixation times were comparable.

The residual formalin fixed tissue was re-examined and an identical set of 26 blocks was taken from both hemispheres to include hippocampal projection sites as well as control regions. Regions included frontal (including the cingulate cortex), temporal, parietal and occipital regions, at a defined level (See appendix A. for block sampling protocol and Figure 4).

The rational for the block sampling is based on current knowledge of known hippocampal projection sites (Duvernoy et al., 2005), including abnormal regions

identified using quantitative MRI on patients with hippocampal sclerosis by Lin et al., (2007) and Cormack et al., (2005) (See Figure 5).

In some cases particular blocks were not available, for example the temporal pole in three out of ten cases had become detached or was not identified in the pre-cut brain. The tissue sampling was carried out together by M.Thom, L.Martinian and F.Blanc. The bocks were then routinely processed and paraffin-wax embedded.

Group	Case	Sex	Age (years)	Fixation Time	Cause of death	Main neuropathology finding
HS	66-04	M	36	4 years	SUDEP	Left HS
	92-94	M	84	14 years	IHD, Pancreatic cancer	Left HS
	55-95	M	74	13 years		Left HS
	45-02	M	74	6 years	Pulmonary Oedema, oesophageal stricture	Bilateral HS
	64-07	M	54	1 year	No neuropathological cause of death	Bilateral HS
	51-06	M	31	2 years	Cause of death unconfirmed	Bilateral HS
Controls	45-98	F	57	10 years	Pancreatitis	Macroscopically normal
	12-98	F	78	10 years	Pancreatic cancer	Macroscopically normal; age related AD pathology
	44-98	F	58	10 years	Myocardial Infarction	Macroscopically normal
	42-97	F	36	11 years	Cardiac arrest	Macroscopically normal

Table 1. Neuropathological details of post-mortem hippocampal sclerosis and control cases
AD= Alzheimer's Disease; HS=hippocampal sclerosis; IHD= ischaemic heart disease; SUDEP= sudden and unexpected death in epilepsy

Case	Left Hippocampal Pathology	Right Hippocampal Pathology	Epilepsy syndrome	Other significant neuropathology
55-95	Classical HS (Unilateral HS)	Mild CA4 gliosis	Encephalitis at 20months caused CPS and GS	Hemiatrophy of left side and cerebellum atrophy. Presence of a few NFTs in entorhinal region
45-02	Classical HS (Bilateral HS)	More pronounced classical HS	Onset at age 14, suffered from GS, OS and CPS.	Hemiatrophy of left hemisphere; laminar neuronal loss from layer III frontal and temporal regions; presence of a few

				AD plaques
66-04	Classical HS with MFS (Unilateral HS)	Normal	MTLE; refractory to treatment	None
92-94	Classical HS (Unilateral HS)	Normal	Onset at age 26; complex partial seizures	Age related AD pathology; few WM vascular changes; mild cerebellar atrophy
64-07	Classical HS with MFS (Bilateral HS)	Resected; residual classical HS	Onset during 20s. Underwent a right temporal lobectomy after which seizues worsened. MRI suggested bilateral damage	Amydala sclerosis; dilated superficial blood vessels (Telangiectasia) in cerebellum
51-06	Classical HS with MFS (Bilateral HS)	Mild end-folium sclerosis	Partial epilepsy	None

Table 2. Additional clinical and pathological details for the HS group
AD= Alzheimer's Disease; CPS= complex partial seizures; GS= generalised seizures; MFS= mossy fibre sprouting; NFTs= neurofibrillary tangles; WM= white matter

3.2. Tissue Preparation

The paraffin-wax embedded sections were cut at 8μm thickness using a microtome and the wax removed via emersion in Xylene for 5 minutes, then 100% and subsequently 70% alcohol for 2 minutes each, washed with water and immersed in distilled water and 3% hydrogen peroxide for a further 15 minutes. After being washed again, the sections were treated with enzymes (Proteinase K, Dakocytomation, Denmark) for 5 minutes in order to open the epitopes, which are closed by the formalin, so that the antibody stains are able to bind to their antigen. Sections were washed with phosphate buffered saline (1:100, Oxoid, Basingstoke, England) before being treated with GFAP (1:1,500; Dakocytomation, Denmark) as well as horseradish peroxidase kit (Dakoenvision) and DAB+ as substrate for visualisation. Sections were further stained with cresyl violet (diluted 1:200). All sections from a single case (a set of 26 slides) were stained in the same batch to control for uniformity of staining. Mrs. Lillian Martinian performed all tissue preparation with F. Blanc as an observer.

Figure 3. (Case 45-02) Evidence of left hemiatrophy in addition to bilateral asymmetrical hippocampal sclerosis

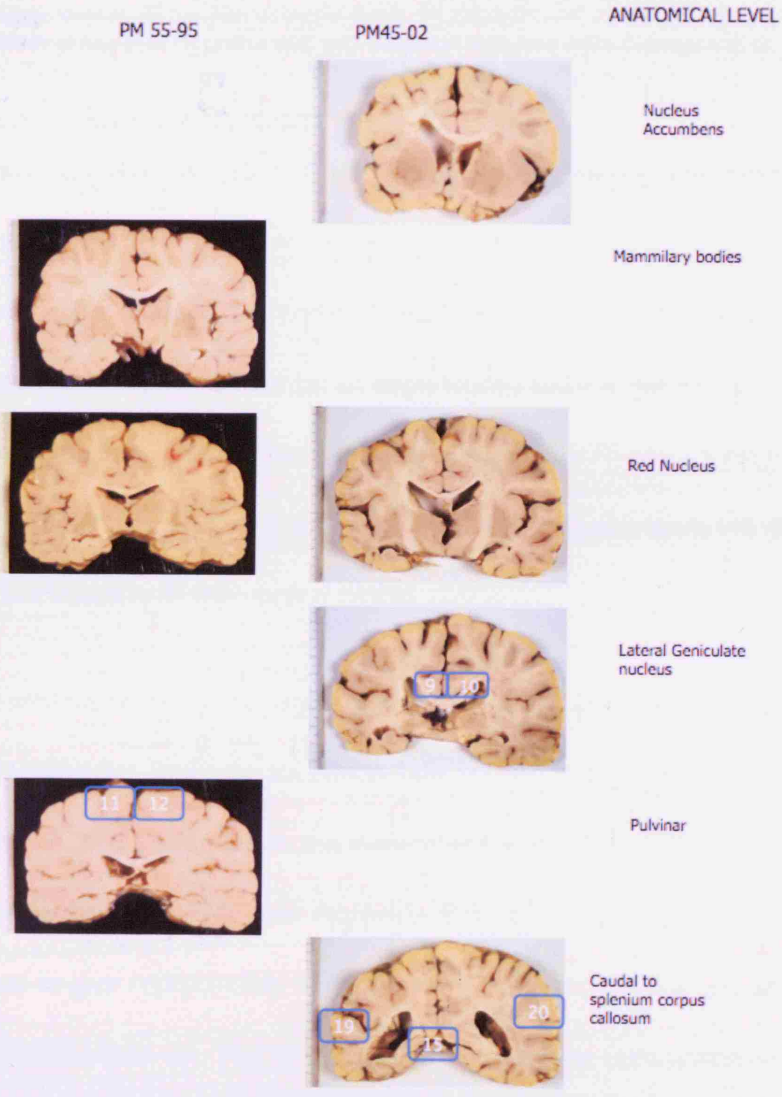

Figure 4. Example of block slicing and anatomical levels used on cases 45-02 and 55-95; 9 & 10- posterior cingulate; 11 & 12- primary motor cortex; 15- inferior retrosplenial cortex; 19 & 20- inferior temporal association cortex.

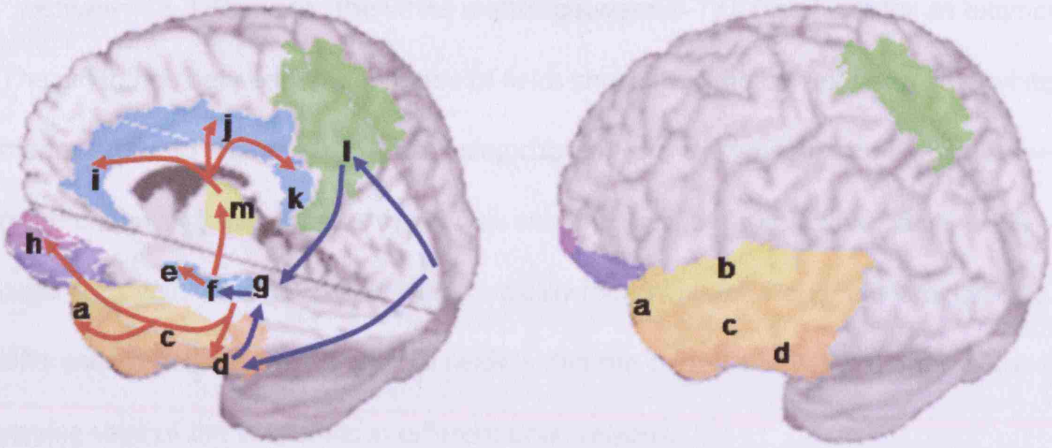

Figure 5. Hippocampal cortical and subcortical afferent (blue) and efferent (red) connections: (a) temporal pole; (b) superior temporal gyrus; (c) middle temporal gyrus; (d) inferior temporal gyrus; (e) amygdala; (f) hippocampus; (g) parahippocampal gyrus; (h) orbitofrontal cortex; (i) anterior, (j) middle, and (k) posterior cingulate gyri; (l) precuneus; (m) thalamus. Adapted from Cormack et al., (2005).

3.3. Analysis of Stained Sections

A commercial image analysis system (Histometrix, Kinetic Imaging, Liverpool, United Kingdom) with a Zeiss Axioskop microscope was used for the field fraction analysis. Two regions of interest (ROI), the cortex (gyral crown) and subcortical white matter were outlined in permanent marker by an experienced pathologist (MT), on each of the slides. This area was then outlined on the image analyser by FB at x2.5 magnification. FB was blind to the case details so did not know whether the cases were HS cases or controls nor the location of the cortical block.

Field fraction analysis relies on staining intensity of regions (Eriksson et al., 2007) and works by calculating the approximate percentage stained in a given area. The percentage of the region analysed in this experiment was set at 20% based on a previously conducted pilot study (see Appendix B for pilot data). 20% sampling of the ROI was found to give reproducible measurements upon resampling and an average coefficient error less than 0.1. The image analysis programme uses uniform random sampling of the fields to be examined at high magnification (x40 objection) within this area. Sampling at 20% meant for the cortex the overall number of fields chosen ranged

between 32-379 and for the white matter between 6-131 (see Fig.6. for an example). The difference between the numbers of fields chosen for the cortex versus the white matter is due to smaller sized areas being chosen from the white matter, which showed more uniform gliosis, whereas the cortex encompassed the gyral crown, a generally larger area with a more variable gliosis typically found in layer 1 and mid-laminar. The difference related to the number of fields within the cortex and white matter is due to varying sizes of the two areas in different brain regions.

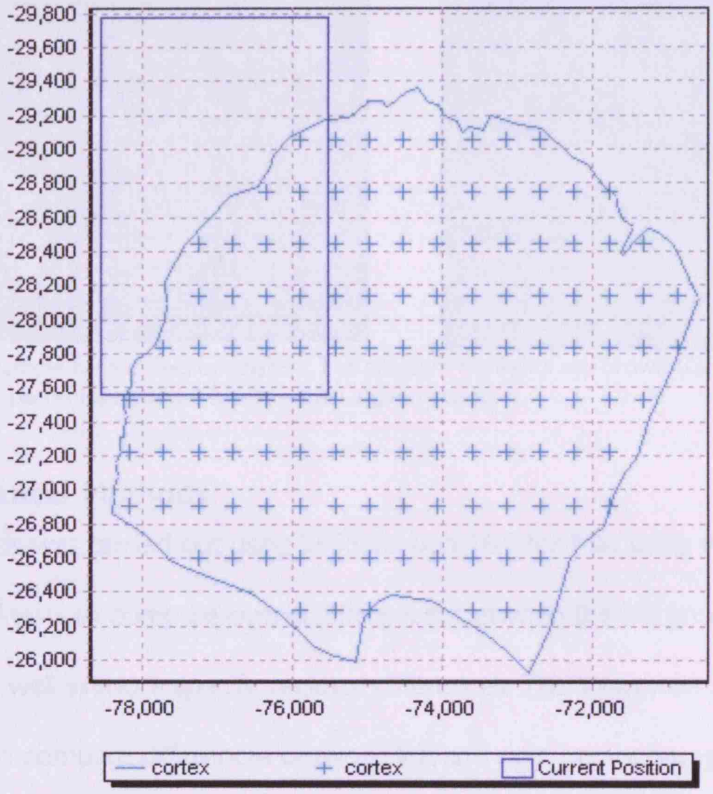

Figure 6. A typical outline of the cortex and the number of fields investigated (127) at 20% sampling using Histometrix. (Case 66-07; Area 2C (prefrontal cortex)).

The RGB (red-green-blue) detection threshold was set for the first field to detect the majority of GFAP immunostaining but minimising non-specific detection. Based on results from the pilot study the programme was 'taught' to recognise GFAP staining at certain intensity (see Fig.7. for an example). The programme then automatically estimates the percentage of GFAP-positive staining in the region and after going

through all of the fields calculates an overall average percentage for each section for that patient. Light intensity and the image analysis RGB (red-green-blue) detection threshold were kept constant throughout the entire experiment for all cases, based on parameters set during the pilot study. The average GFAP stain percentage and the average coefficient error were noted for each region analysed.

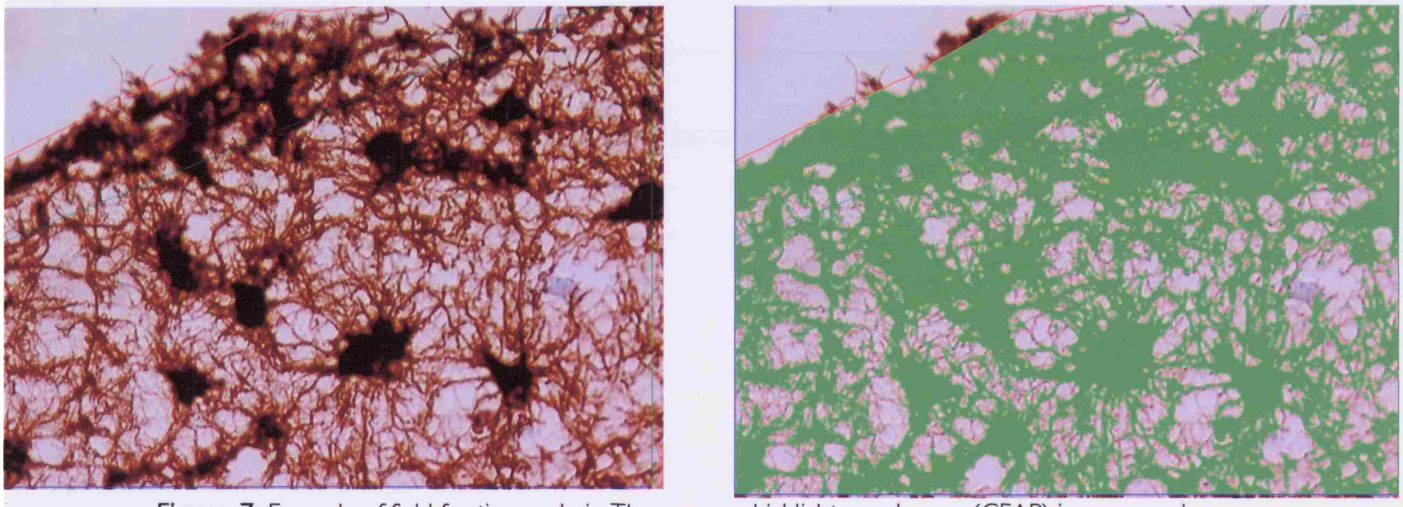

Figure 7. Example of field fraction analysis. The program highlights any brown (GFAP) in green and calculates the percentage stained brown within a given region.

3.4. Statistical Methods

Data analysis was carried out using SPSS version 16.0 for Mac using two-tailed Mann-Whitney *U*-tests to compare overall differences between the HS group and the controls as well as more specific regional differences. The Wilcoxon Signed Ranks Test was used to compare differences between left and right hemispheres of the same cases in order to investigate whether or not there are differences in levels of gliosis in patients who have unilateral or bilateral HS.

4. RESULTS

Qualitative examination of GFAP/CV stained sections showed more uniform and more diffuse patterns of gliosis in the white matter. In the cortex, the most intense GFAP staining was observed in layer 1. The deeper cortex showed more variability of staining intensity. The cytoarchitecture of the cortex matched the area sampled for example; Betz cells were identified in the primary motor cortex.

4.1. Differences between GFAP Percentage in Hippocampal Sclerosis Cases versus Controls in the Cortex and White Matter

The data were not normally distributed and therefore non-parametric statistics were used.

The first analysis involved investigating whether or not there were any overall differences between GFAP percentage in the cortex and white matter of the hippocampal sclerosis cases versus the control cases. Please see Table 3. for mean GFAP percentages averaged across all areas investigated per case.

	Cortex	Average Coefficient Error	White Matter	Average Coefficient Error
Hippocampal Sclerosis Cases				
PM55-95	2.86%	0.21	3.89%	0.13
PM45-02	5.88%	0.14	12.62%	0.10
PM66-04	5.43%	0.12	23.07%	0.03
PM92-94	20.95%	0.06	30.90%	0.04
PM64-07	26.84%	0.04	37.36%	0.03
PM51-06	16.96%	0.07	27.90%	0.04
Overall Average	13.15%	0.11	22.62%	0.06
Control Cases				
PM45-98	4.73%	0.09	12.37%	0.06
PM12-98	8.81%	0.07	11.73%	0.05
PM44-98	4.78%	0.18	13.28%	0.05
PM42-97	0.72%	0.20	1.24%	0.09
Overall	4.76%	0.13	9.66%	0.06

Average				

Table.3. Mean GFAP-positive staining in all cortical and white matter regions studied for each case used with average coefficient errors.

The Mann-Whitney *U*-test conducted showed there to be no significant difference in GFAP percentage staining between the cases with HS and the controls in both the cortex ($z = -1.492$, $p = 0.171$) and white matter ($z = -1.706$, $p = 0.114$). Therefore, there was no significant difference in gliosis between the HS cases and the controls overall.

4.2. Regional and Hemispheric differences between GFAP Percentage in Hippocampal Sclerosis Cases versus Controls

Statistical analyses were conducted in order to compare overall differences between the HS cases and controls in terms of levels of gliosis within specific hippocampal projection regions in both the cortex and white matter. Further investigation was also done to assess whether or not there were any statistical differences between the hemisphere(s) with the sclerosed hippocampus and both of the control cases' hemispheres. This was conducted to ascertain whether the side with HS was more gliotic compared to the control cases.

4.2.1. Bilateral regional differences between GFAP percentage staining in Hippocampal Sclerosis cases versus Controls in the Cortex and White Matter

Comparisons between levels of gliosis in HS cases versus controls in known hippocampal projection regions were made using the Mann-Whitney *U*- Test in order to investigate whether some regions were more or less affected than others, as has been stipulated in the neuroimaging research papers mentioned in chapter 2.4. Areas were selected on the basis of receiving projections from the hippocampus as well as being highlighted as being affected by more widespread change by the neuroimaging research. Please see Tables 4 (cortical areas) and 5 (white matter areas) for chosen

areas and results. The same regions were selected for both the cortex and white matter.

Region	Total number. of HS sections (bilateral)	Mean GFAP staining in HS subjects	Total number of control sections (bilateral)	Mean GFAP staining in controls	Z	*P* value
Prefrontal Cortex	12	15.06%	7	4.96%	-2.451	**0.013**
Anterior Cingulate	12	14.22%	8	7.29%	-0.926	0.384
Posterior Cingulate	10	11.99%	8	4.49%	-1.866	0.068
Superior Retrosplenial	10	13.32%	8	3.78%	-1.777	0.083
Inferior Retrosplenial	8	10.31%	8	6.70%	-0.735	0.505
Superior Parietal	12	14.30%	8	4.64%	-1.929	0.057
Occipito-temporal area 37	12	11.97%	8	4.17%	-1.852	0.069

Table 4. GFAP percentage staining in indicated regions within the cortex of HS cases and controls, including the number of bilateral sections available for both groups. Bolded P-values indicate significance at $p < 0.05$.

The prefrontal cortices of the HS cases, within the cortex, were found to have significantly more GFAP stain, i.e. more gliosis than that of the control cases ($z=-2.451$, $p=0.01$). No other significant differences were found for the cortex.

Region	Total number of HS sections (bilateral)	Mean GFAP staining in HS subjects	Total number of control sections (bilateral)	Mean GFAP staining in controls	Z	*P* value
Prefrontal Cortex	12	20.00%	7	10.13%	-2.028	**0.045**
Anterior Cingulate	11	20.85%	8	9.34%	-2.147	**0.033**
Posterior Cingulate	10	23.07%	8	7.91%	-2.577	**0.009**
Superior Retrosplenial	10	20.49%	8	5.66%	-2.044	**0.043**
Inferior Retrosplenial	8	26.86%	8	16.34%	-1.576	0.130
Superior Parietal	12	21.03%	8	7.68%	-2.006	**0.047**
Occipito-temporal area 37	12	22.54%	8	8.81%	-2.315	**0.020**

Table 5. GFAP percentage staining in indicated regions within the white matter of HS cases and controls, including the number of bilateral sections available for both groups. Bolded *P*-values indicate significance at $p < 0.05$.

Significant differences between GFAP staining in the HS cases and controls within the white matter regions of the prefrontal cortex (z=-2.028, p=0.045), anterior cingulate (z= -2.147, p= 0.03), posterior cingulate (z= -2.577, p= 0.009), superior retrosplenial (z= -2.044, p=0.04), superior parietal (z= -2.006, p=0.047) and occipitotemporal area (z= -2.315, p=0.02) were found at p=<0.05. There were no significant differences in GFAP staining within the inferior retrosplenial cortex between the HS cases and the controls.

4.2.2. Hemispheric differences in GFAP percentage staining between the sclerosed Hippocampal Hemisphere of HS Cases and Control Cases of Hippocampal Projection Regions

The GFAP percentages for each chosen hippocampal projection region in the sclerosed hemisphere of the HS cases, which were either unilateral or bilateral, were combined and compared with the bilateral GFAP percentage values of the control cases. The non-HS hemisphere from the unilateral cases was not included.

The mean GFAP percentage in the affected hemispheres of all cases in the HS group, from the cortex, was 14.48% (mean coefficient error 0.09) compared with the mean GFAP percentage of all the control hemispheres, which was 5.14% (mean coefficient error 0.14). A Mann-Whitney *U*-Test found the difference to be significant (z= -5.046, p=0.00) and therefore the hemisphere(s) with HS from the HS cases showed significantly more cortical gliosis than the control hemispheres.

In the white matter, the mean GFAP staining percentage in the hippocampal projection regions was 23.62% for the HS group (mean coefficient error 0.05) and 9.43% (mean coefficient error 0.06) in the control group. A Mann-Whitney *U*-Test found the difference to be significant (z= - 5.563, p=0.00) so it can be concluded that the

hemisphere(s) with HS from the HS cases showed significantly more white matter gliosis than the control hemispheres.

4.3. Within-subject Hemispheric differences in Cases with Unilateral or Bilateral Hippocampal Sclerosis

As well as investigating between group differences, we wished to investigate whether or not there were any within subject differences: namely whether or not there were different patterns of gliosis in unilateral HS or bilateral HS cases, such as one hemisphere being more affected than the other. This was done using the Wilcoxon signed-rank test. Please see Table 6. below for obtained results.

Case	Affected Hemisphere	Region	Mean GFAP staining in left hemisphere	Mean GFAP staining in right hemisphere	Z	*P* value
PM55-95	Left CHS Right EFG	Cortex	3.47%	2.19%	-0.078	0.937
		White Matter	5.11%	2.68%	-0.622	0.534
PM92-94	Left CHS Right EFG	Cortex	19.31%	22.59%	-1.956	**0.05**
		White Matter	32.30%	29.63%	-1.070	0.285
PM66-04	Left CHS Right EFG	Cortex	5.69%	5.17%	-1.255	0.209
		White Matter	21.63%	24.65%	-1.156	0.248
PM45-02	Bilateral HS Left & Right CHS	Cortex	6.43%	5.29%	-0.978	0.328
		White Matter	15.29%	9.67%	-1.580	0.114
PM64-07	Bilateral HS Left & Right CHS	Cortex	24.59%	29.09%	-2.197	**0.028**
		White Matter	36.66%	38.06%	-0.549	0.583
PM51-06	Bilateral HS Left CHS & Right EFS	Cortex	17.26%	16.66%	-0.445	0.657
		White Matter	30.03%	25.77%	-0.800	0.424

Table 6. Comparison of GFAP percentage staining between the left and right hemispheres in HS cases. The HS hemisphere is indicated and bolded *P*-values indicate significance at $p < 0.05$.
CHS= Classical hippocampal sclerosis; EFG= End folium gliosis, EFS= End folium sclerosis; HS= Hippocampal sclerosis

Only two cases, PM92-94 and PM64-07 were found to have significant differences in percentage GFAP stain between the left and right hemispheres. PM92-94, with left HS, was found to have significantly more gliosis in the right hemisphere than the left ($z = -1.956$, $p = 0.05$) and PM64-07, with bilateral HS, was also found to have significantly

more gliosis in the right hemisphere (z= -2.197, p= 0.028). Both differences were only found in the cortex. There were no other significant hemispheric differences within subjects.

4.4. Regional Hemispheric differences in Cases with Unilateral or Bilateral Hippocampal Sclerosis

Specific regional hemispheric differences were investigated as well in subjects by comparing hippocampal projection areas in the hemisphere lateral to the HS against the hemisphere with no HS. This was conducted by combining the results from the hippocampal projection regions of all the HS cases and investigating the difference in gliosis between the hemisphere with HS, be that unilateral or bilateral against the hemisphere without HS. This meant that 6 hemispheres were compared against 3, as 3 of the cases had bilateral HS.

In the cortex, the mean GFAP percentage staining in the hippocampal projection regions lateral to the sclerosed hippocampus was 14.48% (mean coefficient error 0.09). The mean GFAP staining in the regions contralateral was 9.48% (mean coefficient error 0.14). A Mann-Whitney *U*-Test conducted showed the difference to be significant (z= -2.20, p=0.028) and therefore the hippocampal projection areas in the hemisphere lateral to the HS were more gliotic than the contralateral hemisphere.

In the white matter, the mean GFAP percentage staining in the hippocampal projection regions lateral to the sclerosed hippocampus was 23.62% (mean coefficient error 0.05) whereas the mean GFAP staining in the regions contralateral to the sclerosed hippocampus was 16.52% (mean coefficient error 0.07). A Mann-Whitney *U*- Test showed this difference to be significant (z= -1.949, p= 0.05) therefore the white matter of hippocampal projection areas in the hemisphere lateral to the HS were more gliotic than the non-HS side.

5. DISCUSSION

Hippocampal sclerosis is the commonest pathology in patients undergoing surgery for refractory TLE (Thom et al., 2002) and is found in 50-75% of such patients (Jefferys, 1999). Whilst over half of patients become completely seizure free at least 20-30% continue to experience seizures after surgery (Wyler et al., 1995; Wieser et al., 2003; Özkara et al., 2008) and many will remain on anti-epileptic medications. This suggests that the hippocampus may not be the only source of focal seizures in such patients but actually extra-hippocampal and extra-temporal structures may be involved. Extra-hippocampal damage in TLE has been long recognised in surgical tissues (Cavanagh & Meyer, 1956) however recent quantitative neuroimaging studies have suggested that more extensive extra-temporal damage may be occurring.

Quantitative neuroimaging research conducted by Lin et al., (2007), Cormack et al., (2006) and Thivard et al., (2005) suggest that TLE patients with HS infact have even more widespread cortical abnormalities, although their pathological basis is unknown. This is the first quantitative neuropathological analysis carried out on post mortem tissues from patients with epilepsy and HS to investigate the nature, severity and distribution of any extra-hippocampal cortical and white matter abnormalities.

5.1. Main Findings and Interpretation

Epilepsy post mortems from patients with bi- and unilateral HS were selected and 26 cortical regions analysed per case according to information from quantitative neuroimaging studies regarding potential sites of atrophy. The qualitative inspection of the sections showed variable gliosis between and within cases. Quantitative analysis of GFAP was carried out as a measure of cortical astrogliosis and an indicator of pathology and atrophy.

We investigated whether specific regional differences existed i.e. if particular brain areas that received hippocampal projections were more gliotic in the HS cases than the controls and furthermore if there was a difference in gliosis between the hemispheres ipsilateral versus those contralateral to the sclerosed hippocampus.

Within the cortex, a significant difference between the amount of GFAP in the prefrontal cortex between the HS cases and the controls were found, namely the HS cases had a significantly higher amount of GFAP present and therefore more gliosis. Other than here, no significant differences were found for the other regions.

Within the white matter, several regions were significantly affected in the HS cases versus the controls, these being the prefrontal cortex, anterior and posterior cingulate, the superior retrosplenial cortex, the superior parietal lobule and occipitotemporal area 37. It can be concluded that the aforementioned areas from the white matter, as well as the cortical prefrontal cortex were more gliotic in the HS cases than the controls.

The neuroimaging research discussed in chapter 2 suggested that several cortical hippocampal projection regions were more atrophic in TLE patients with HS however we have only found the prefrontal cortex to be significantly more gliotic than the control group.

Upon investigating the amount of GFAP present in the hippocampal projection regions ipsilateral and contralateral to the sclerosed hippocampus between the two groups it was found that there was a significant difference at $p<0.01$ for both the cortex and the white matter. Therefore the chosen hippocampal projection regions had significantly more gliosis in the hemisphere ipsilateral to the HS than the normal control hemispheres. Further investigation of the hippocampal projection regions ipsilateral and contralateral to the sclerosed hippocampus using the HS cases alone showed that when the HS was ipsilateral there was significantly more gliosis in the hippocampal projection regions than on the contralateral side.

Within-subjects investigations were also carried out to determine whether there were different patterns of gliosis in the unilateral and bilateral HS cases, such as one hemisphere being more affected than the other. Only 2 significant results were found here. One case with left HS actually had significantly more gliosis on the contralateral side within the cortex and another with bilateral HS also had significantly more gliosis in the right hemisphere, which was also within the cortex, thus suggesting the overall brain is affected in both hemispheres. Though as found in the previous analysis, the ipsilateral hippocampal projection regions are more affected than the contralateral ones. No significant differences were found for values obtained from the white matter suggesting that it is uniformly affected regardless of the laterality of the HS. This could be suggestive of generalised seizures perhaps, which affect both cerebral hemispheres. On the other hand the cases chosen were a particularly refractory population who may have had other non-neurological conditions such as hypertension, so care must be taken when interpreting these results.

Furthermore and perhaps paradoxically the cases with visible macroscopic hemiatrophy (45-02; 55-95) were not the ones with the highest overall GFAP scores and did not show significant asymmetry in GFAP staining intensity in the inter-hemispheric analysis, which would have been expected. It is possible then that noticeable atrophy does not necessarily correlate with level of gliosis but may have a better relationship with other markers such as neuronal loss or microglia activation.

No significant differences were found in overall mean GFAP percentage staining in the cortex and white matter between HS cases and the control cases, when values from both hemispheres were combined, therefore there was no significant difference between overall levels of gliosis in the two groups. However, it should not be prematurely concluded that no difference at all exists. The HS group did have a higher

overall mean GFAP percentage staining, especially within the white matter. It is likely that the small sample size acted as a confound, due to there being only 6 HS cases and 4 control cases. The limitations of the study will however be discussed more in-depth later in this chapter.

A clear finding from this study is that when a selection of hippocampal projection regions are separated, they appear to be more markedly affected. This correlates nicely with previous findings of Lin et al., (2007), who showed, using refractory surgical MTLE patients with HS, up to 30% bilateral decreases in cortical thickness within the frontal poles, lateral temporal and occipital regions and Cormack et al., (2005), who found, using paediatric TLE patients with MTS, significant grey matter density reduction within the lateral temporal lobes, posterior cingulate and retrosplenial cortex. Furthermore, the white matter in our series is just as, if not more, adversely and diffusely affected. It has been noticed in diffusion tensor imaging (DTI) studies, such as Concha et al., (2007) that even after epilepsy surgery white matter abnormalities persist, even though axonal/myelin damage is expected to be minimal (Concha et al., 2007). Nevertheless, Concha et al., (2007) report that white matter abnormalities appear to have no influence in relation to seizure freedom in TLE post-surgical patients.

There may be several causes pertaining to the presence of more gliosis in hippocampal projection regions. If seizure generation is due to HS, it is possible that this activity spreads to its efferent connections, which degenerate over time due to repeated seizure exposure. It is also possible that these structures may themselves become epileptogenic and can produce seizures in the absence of the primary epileptic focus, a phenomena known as secondary epileptogenesis (Morrell, 1985; Salanova et al., 1996). Thus even in the absence of the sclerosed hippocampus, due to the area being

resected, regions formally connected to the structure could continue to produce seizures. If this is the case, this does assist with beginning to understand why surgery is not always successful. Furthermore, it is more likely that with repeated exposure to seizure activity structures become more adversely affected and thus patients who have endured their epilepsy for a long time may be less likely to have successful surgical outcome in terms of seizure freedom. Janszky et al., (2005) and Yoon et al., (2003) show that pre-surgical seizure durations of 15 years or more is strongly associated with seizure recurrence 5 years after surgery for TLE. It is therefore imperative for those with intractable MTLE to be treated as soon as possible. A pre-surgical presence of secondary generalised tonic-clonic seizures and ictal dystonia have also been found to have associations with recurrent seizures after 2 post-operative years (Janszky et al., 2005). It is also clear that the hemisphere ipsilateral to the HS has more markedly gliotic projection regions.

The exact cellular mechanisms related to intractable TLE and reasons as to why surgery is not always successful are currently unknown. Although neuron dysfunction is often the main focus of seizure generation, glial cells, especially astrocytes, may play a very important role in excitability generation at the foci of seizures (Jabs et al., 2008; Binder & Steinhäuser, 2006; Chang & Lowenstein, 2003).

Astrocytes are mainly responsible for glutamate uptake (Jabs et al., 2008) and glutamate transporters and receptors have been implicated in the development and spread of seizures. Eid et al., (2008) postulate that excess concentrations of glutamate in the sclerotic hippocampus may be one of the key molecular causes of seizures and subsequent neuronal damage in TLE. Furthermore, the loss of the enzyme, glutamine synthetase in MTLE patients (Eid et al., 2004), which converts glutamate into glutamine, offers a possible explanation into how excess glutamate may build up within the

hippocampus. In addition, downregulation or dysfunction of glutamate transporters within the hippocampus has also been suggested to be either a cause, or a consequence, of seizures (Proper et al., 2002). It is possible then that in TLE, structures connected to the hippocampus also experience excitotoxic glutamate levels or disruptions in glutamate transport. Studies that have explored changes in glutamate concentration, transport or receptors in TLE have tended to use resected tissue (Notenboom et al., 2006) or concentrate on the hippocampus and temporal neocortex (van der Hel, 2005; Eid 2004) so it is unknown whether or not such changes are seen in TLE patients that have HS in other brain regions.

There may also be abnormal neurodevelopmental factors here, such as microscopic malformations of cortical development, which occur in approximately 40% of MTLE patients with HS (Guerrini et al., 2003). This means that subtle malformations could exist in non-resected neocortical tissue and these are known to have associations with lowered seizure thresholds. Indeed Ho et al., (1998) showed that in a group of patients with temporal lobe development malformations, those with pure HS had a higher frequency of hippocampal formation atrophy and amygdala atrophy, suggesting that dual pathology, i.e. MTS associated with temporal lobe development malformations, are not uncommon in TLE patients.

Neuropathological features of cortical dysplasia include the presence of balloon cells, dysmorphic neurones, hypertrophic pyramidal neurones, grey matter heterotopia and neuronal glial clustering (Thom 2007; Lin et al., 2007; Prayson & Frater, 2003). It may be interesting to investigate whether such pathology is present in other regions, such as the hippocampal projection areas used in this study and whether there is any relationship between developmental cortical malformations and the presence of neuronal damage

away from the hippocampus. It should be clarified however that in the cases used in this study, there was no histological evidence of cortical malformations.

5.2. Limitations of the Study

As with all research, this study had various limitations. The sample size as mentioned in 5.1. was fairly small due to time limitations and the examination of extensive regions per case. This increases the likelihood of making a Type II error: that is failing to reject the null hypothesis when in fact a difference does exist. Looking at the mean overall GFAP percentages for the epilepsy/HS cases against the controls shows that there is a difference between the two groups, ones which may be found to be significant with further study using larger sample sizes. This study should however be regarded as pilot research as it the first to address the issue of widespread changes in TLE cases via quantitative post-mortem research.

We wished to explore possible explanations concerning why some patients who undergo resective surgery may not always be relieved of their seizures. The cases used in this study make it difficult to draw direct comparisons to surgical patients for various reasons. Firstly, the HS cases here may be less homogenous than patients chosen for surgery due to different clinical histories, different causes of their epilepsy as well as the presence of bilateral HS (patients chosen for surgery usually have unilateral HS). Furthermore, surgery is found to be more successful in younger patients who have had a shorter duration of epilepsy. The cases in this study tend to be older and thus also have the problem of other confounding pathologies. However, effort was made to exclude cases with neurodegenerative disease, those who had suffered from stroke as well as those who displayed minor head injuries as a result of seizures. Furthermore, Janszky et al., (2005) highlight that surgery is less successful in older patients with longer

epilepsy duration, which are the type of cases used in this study. Therefore limited success with such patients may be due to increased involvement of extra-temporal structures as hypothesised here.

Surgical cases also have the advantage of MRI data for comparison whereas none was available here for any of the cases.

The brains used were all pre-cut archival samples and although every effort was made to include all 26 chosen cortical regions, in some cases there was insufficient wet residual tissue and in others it was difficult, if not impossible, to distinguish between the left and right hemisphere due to the tissue being reduced in size and fragmented. Furthermore, there were differences between the fixation times, which ranged from 1-14 years, which could have affected staining between the cases. This could be assessed adapting a method employed by Sharma & Grieve (2006), who selected a number of post-mortem brains between the years 1998-2001 and employed an independent assessor to score staining quality after the tissue had been fixed. On the other hand, one case, PM92-94 who had the longest fixation time, showed high GFAP field fractions so it is unlikely that fixation time was a confound. Furthermore, era appropriate controls were selected whenever possible to make the comparison more reliable. The mean ages of both groups were fairly similar with the epilepsy group having a mean age of 54 years and the controls a mean age of 57 years.

We have also neglected to explore the possible effects that antiepileptic drugs may have on pathology as previous studies have found that they may also be responsible for tissue loss (Sandok et al., 2000).

Lastly, we did not correct for multiple comparisons, which may affect some conclusions drawn here.

5.3. Suggestions for Further Study

It would be interesting to explore different markers, such as CD68, which marks microglia activation, to investigate whether or not gliosis is the only indicator of widespread change. Neuronal loss could also be explored, though perhaps using stereological techniques would be more suitable for this.

A further PM study should obtain a larger sample size and include cases with left and right MTLE. Bonilha et al., (2007) suggest that there are differences between extra-hippocampal atrophy, which correlate with the HS hemisphere and therefore this idea could be further explored using PM tissue. It would be useful to corroborate PM findings with MRI data, which we were not able to do in this study. Correlations between age at onset of epilepsy and the amount of gliosis/atrophy present would also be useful.

A longitudinal study would be ideal as a group of surgical TLE patients could be followed post-surgery up until the time of death and thus if some were continuing to experience seizures whereas others declined, investigation of their PM tissue may elucidate reasons as to why this could occur. Such research could also aim to correlate findings with patient's psychological status as it has been documented that individuals with intractable MTLE experience cognitive decline over time (Bonilha et al., 2007; Cendes, 2005). Indeed, in this study it was found that the prefrontal cortex was one of the more gliotic cortical areas and this region is well known to be involved in a range of cognitive functions, including executive functioning, planning complex behaviours and so on.

5.4. Conclusion

HS is the most common pathology seen in MTLE patients. Patients with intractable MTLE may undergo surgery in order to be relieved of their seizures however up to

30% of patients may continue to experience post-operative seizures suggesting that the epileptogenic location may not be as focal as previously thought. In this study we have shown that hippocampal projection regions are significantly more gliotic in post-mortem tissue from HS patients compared to that of controls, paralleling recent neuroimaging research, which has found hippocampal projection structures to be more atrophic in patients with MTLE and HS. Further study may wish to correlate levels of gliosis with seizure duration and quantitiative MRI data as well as explore possible cellular and neurodevelopmental hypotheses to aid explanation as to why other brain regions are being affected by focal seizure activity.

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TABLES AND FIGURES

APPENDIX A

- Example of block taking sheet used

HS: Cortical Study BLOCK SHEET
Case Number: Name:

# L R	Region	Block	Coronal Level	Left Block no.	Right Block no.
FRONTAL					
1 2	Pre-frontal cortex (Pole) Area 10		Most anterior coronal slice	1c	2c
3 4	Orbito Frontal Area 11	Rectus Gyrus (both sides of olfactory sulcus)	Anterior to ventricle	3c	4c
CINGULATE					
5 6	Anterior (Area 24)	Cingulate	Level Nucleus accumbens	5c	6c
9 10	Posterior (Area 23)	Cingulate	Level LGN	9c	10c
13 14	Retrosplenial (Area 29/30)	Cingulate (Superior CC)	Splenium CC	13c	14c
15 16	As above	Gyrus inferior to CC (include caudal HC)	Splenium CC	15c	16c
PARIETAL					
17 18	Post parietal association cortex (Area 7)	Sup/paramedian	Splenium of CC	17c	18c
TEMPORAL					
19 20	Inferior temporal association cortex (Area 37)	Post STG	Splenium CC: after most caudal extent STG	19c	20c
25 26	Temporal pole (Area 38)	Pole: STG	Temporal lobe pole	25c	26c
OCCIPITAL					
21 22	Area 39	IPLobule	Caudal extent of left ventricle	21c	22c
23 24	Area 17 (control) Calcarine striate cortex	Visual cortex	As above	23c	24c
OTHER BLOCKS					
7 8	Primary Sensory	F1/F2	Level mamillary bodies	7c	8c
11 12	Primary motor	Paracentral gyrus	Caudal pulvinar/LGN	11c	12c

CC= corpus callosum; LGN= lateral geniculate nucleus; IPLobule= inferior parietal lobule; STG=superior temporal gyrus

APPENDIX B

- Pilot study data. Obtained from case 45-02, area 1C (left pre-frontal cortex)

Trial (Cortex)	Sampling Percentage	Number of Fields	GFAP staining (%)	Average Coefficient Error
1	100%	642	25.44%	0.0267
	50%	322	11.04%	0.0673
	20%	130	19.43%	0.0656
	10%	66	18.4%	0.1066
2	100%	642	14.75%	0.0292
	50%	322	14.1%	0.0417
	20%	130	17.28%	0.0815
	10%	66	14.99%	0.0892
3	100%	642	11.4%	0.0348
	50%	322	11.54%	0.0485
	20%	130	12.06%	0.0815
	10%	66	11.8%	0.1029
4	100%	642	11.86%	0.0343
	50%	322	12.06%	0.0506
	20%	130	11.38%	0.0778
	20%	66	12.7%	0.1103
Trial (White Matter)				
1	100%	215	21.02%	0.0149
	50%	109	21.33%	0.0242
	20%	44	20.54%	0.0394
	10%	22	21.61%	0.0348
2	100%	215	21.09%	0.0155
	50%	109	20.52%	0.0228
	20%	44	21.74%	0.035
	10%	22	20.97%	0.0329

APPENDIX C

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM55-95	**Prefrontal Cortex- Pole**	Cortex	6.00%	0.189	83
PM55-95	**Area 10**	Cortex	2.45%	0.277	95
PM55-95	Orbitofrontal Cortex	Cortex	3.77%	0.205	94
PM55-95	Rectus Gyrus	Cortex	4.07%	0.172	139
PM55-95	**Anterior Cingulate (24)**	Cortex	2.15%	0.171	86
PM55-95	**Anterior Cingulate (24)**	Cortex	4.06%	0.221	77
PM55-95	Primary Sensory cortex	Cortex	2.00%	0.166	140
PM55-95	Primary Sensory cortex	Cortex	1.73%	0.25	105
PM55-95	**Posterior Cingulate (23)**	Cortex	2.60%	0.252	76
PM55-95	**Posterior Cingulate (23)**	Cortex	2.70%	0.31	77
PM55-95	PMC-paracentral gyrus	Cortex	2.03%	0.681	114
PM55-95	PMC-paracentral gyrus	Cortex	1.44%	0.172	100
PM55-95	**Retrosplenial (29/30- Superior CC**	Cortex	0.86%	0.085	99
PM55-95	**Retrosplenial (29/30- Superior CC**	Cortex	1.56%	0.328	54
PM55-95	**Retrosplenial (29/30- Inferior CC**	Cortex	2.53%	0.12	72
PM55-95	**Retrosplenial (29/30- Inferior CC**	Cortex	0.94%	0.17	71
PM55-95	**Superior Parietal**	Cortex	0.67%	0.079	105
PM55-95	**Superior Parietal**	Cortex	0.93%	0.144	89
PM55-95	**Occipitotemporal**	Cortex	2.60%	0.205	123
PM55-95	**Occipitotemporal**	Cortex	0.73%	0.07	89
PM55-95	Angular	Cortex	0.69%	0.111	100
PM55-95	Angular	Cortex	3.05%	0.22	78
PM55-95	V1	Cortex	2.03%	0.218	77
PM55-95	V1	Cortex	2.60%	0.277	65
PM55-95	Temporal Pole	Cortex	17.24%	0.082	94
PM55-95	Temporal Pole	Cortex	n/a	n/a	n/a
		Average	2.86%	0.207	92.08
					140
					54

PM55-95	**Prefrontal Cortex- Pole**	White Matter	1.12%		0.082		73
PM55-95	**Area 10**	White Matter	1.82%		0.072		60
PM55-95	Orbitofrontal Cortex	White Matter	2.12%		0.072		58
PM55-95	Rectus Gyrus	White Matter	3.40%		0.058		64
PM55-95	**Anterior Cingulate (24)**	White Matter	8.27%		0.066		30
PM55-95	**Anterior Cingulate (24)**	White Matter	3.17%		0.097		33
PM55-95	Primary Sensory cortex	White Matter	1.23%		0.685		63
PM55-95	Primary Sensory cortex	White Matter	2.60%		0.168		25
PM55-95	**Posterior Cingulate (23)**	White Matter	3.53%		0.075		47
PM55-95	**Posterior Cingulate (23)**	White Matter	6.37%		0.065		88
PM55-95	PMC-paracentral gyrus	White Matter	1.20%		0.161		21
PM55-95	PMC-paracentral gyrus	White Matter	3.09%		0.061		43
PM55-95	**Retrosplenial (29/30- Superior CC**	White Matter	1.98%		0.073		48
PM55-95	**Retrosplenial (29/30- Superior CC**	White Matter	1.56%		0.16		26
PM55-95	**Retrosplenial (29/30- Inferior CC**	White Matter	20.00%		0.076		32
PM55-95	**Retrosplenial (29/30- Inferior CC**	White Matter	3.83%		0.121		23
PM55-95	**Superior Parietal**	White Matter	1.63%		0.087		38
PM55-95	**Superior Parietal**	White Matter	0.94%		0.111		22
PM55-95	**Occipitotemporal**	White Matter	3.70%		0.126		35
PM55-95	**Occipitotemporal**	White Matter	1.26%		0.149		37
PM55-95	Angular	White Matter	n/a	n/a		n/a	
PM55-95	Angular	White Matter	1.40%		0.124		37
PM55-95	V1	White Matter	11.38%		0.095		36
PM55-95	V1	White Matter	n/a	n/a		n/a	
PM55-95	Temporal Pole	White Matter	n/a	n/a		n/a	
PM55-95	Temporal Pole	White Matter	n/a	n/a		n/a	
		Average	3.89%		0.126		42.68
							88
							21

Identity	Area	Region	Vf	Coeffiecient Error	No. Of Fields
PM45-02	**Prefrontal Cortex- Pole**	Cortex	12.51%	0.078	131
PM45-02	**Area 10**	Cortex	7.37%	0.127	72
PM45-02	Orbitofrontal Cortex	Cortex	12.72%	0.127	51
PM45-02	Rectus Gyrus	Cortex	6.63%	0.2	63
PM45-02	**Anterior Cingulate (24)**	Cortex	5.33%	0.131	99
PM45-02	**Anterior Cingulate (24)**	Cortex	3.06%	0.142	146
PM45-02	Primary Sensory cortex	Cortex	6.58%	0.162	111
PM45-02	Primary Sensory cortex	Cortex	4.95%	0.171	117
PM45-02	**Posterior Cingulate (23)**	Cortex	n/a	n/a	n/a
PM45-02	**Posterior Cingulate (23)**	Cortex	n/a	n/a	n/a
PM45-02	PMC-paracentral gyrus	Cortex	5.59%	0.116	124
PM45-02	PMC-paracentral gyrus	Cortex	3.05%	0.216	104
PM45-02	**Retrosplenial (29/30- Superior CC**	Cortex	5.30%	0.141	96
PM45-02	**Retrosplenial (29/30- Superior CC**	Cortex	1.91%	0.121	134
PM45-02	**Retrosplenial (29/30- Inferior CC**	Cortex	5.50%	0.111	88
PM45-02	**Retrosplenial (29/30- Inferior CC**	Cortex	5.11%	0.106	114
PM45-02	**Superior Parietal**	Cortex	3.02%	0.188	83
PM45-02	**Superior Parietal**	Cortex	4.48%	0.118	115
PM45-02	**Occipitotemporal**	Cortex	5.41%	0.144	131
PM45-02	**Occipitotemporal**	Cortex	6.37%	0.129	120
PM45-02	Angular	Cortex	2.94%	0.227	105
PM45-02	Angular	Cortex	7.21%	0.142	91
PM45-02	V1	Cortex	3.87%	0.144	90
PM45-02	V1	Cortex	8.05%	0.125	50
PM45-02	Temporal Pole	Cortex	8.36%	0.106	62
PM45-02	Temporal Pole	Cortex	n/a	n/a	n/a
		Average	5.88%	0.142	99.87
					146
					50

PM45-02	**Prefrontal Cortex- Pole**	White Matter		20.56%		0.034		44
PM45-02	**Area 10**	White Matter		4.25%		0.141		31
PM45-02	Orbitofrontal Cortex	White Matter		15.85%		0.1		35
PM45-02	Rectus Gyrus	White Matter		7.55%		0.06		40
PM45-02	**Anterior Cingulate (24)**	White Matter		19.84%		0.078		23
PM45-02	**Anterior Cingulate (24)**	White Matter		5.13%		0.052		53
PM45-02	Primary Sensory cortex	White Matter	n/a		n/a		n/a	
PM45-02	Primary Sensory cortex	White Matter		2.39%		0.208		26
PM45-02	**Posterior Cingulate (23)**	White Matter	n/a		n/a		n/a	
PM45-02	**Posterior Cingulate (23)**	White Matter	n/a		n/a		n/a	
PM45-02	PMC-paracentral gyrus	White Matter		15.20%		0.082		30
PM45-02	PMC-paracentral gyrus	White Matter	n/a		n/a		n/a	
PM45-02	**Retrosplenial (29/30- Superior CC**	White Matter		18.00%		0.063		34
PM45-02	**Retrosplenial (29/30- Superior CC**	White Matter		5.15%		0.07		46
PM45-02	**Retrosplenial (29/30- Inferior CC**	White Matter		31.37%		0.045		35
PM45-02	**Retrosplenial (29/30- Inferior CC**	White Matter		10.93%		0.307		11
PM45-02	**Superior Parietal**	White Matter		4.81%		0.281		20
PM45-02	**Superior Parietal**	White Matter		11.91%		0.056		47
PM45-02	**Occipitotemporal**	White Matter		3.34%		0.092		76
PM45-02	**Occipitotemporal**	White Matter		15.56%		0.076		53
PM45-02	Angular	White Matter		2.32%		0.133		46
PM45-02	Angular	White Matter		13.11%		0.07		27
PM45-02	V1	White Matter		15.97%		0.065		36
PM45-02	V1	White Matter		20.70%		0.04		37
PM45-02	Temporal Pole	White Matter		20.98%		0.052		44
PM45-02	Temporal Pole	White Matter	n/a		n/a		n/a	
		Average		12.62%		0.1		37.81
								76
								11

Identity	Area	Region	Vf	Coefficient Error	No. of Fields
PM66-04	Cortex	**Prefrontal Cortex- Pole**	4.03%	0.125	161
PM66-04	Cortex	**Area 10**	3.98%	0.14	127
PM66-04	Cortex	Orbitofrontal Cortex	5.11%	0.148	126
PM66-04	Cortex	Rectus Gyrus	4.62%	0.089	226
PM66-04	Cortex	**Anterior Cingulate (24)**	4.23%	0.126	173
PM66-04	Cortex	**Anterior Cingulate (24)**	4.43%	0.132	195
PM66-04	Cortex	Primary Sensory cortex	4.64%	0.088	222
PM66-04	Cortex	Primary Sensory cortex	4.62%	0.134	145
PM66-04	Cortex	**Posterior Cingulate (23)**	6.14%	0.147	84
PM66-04	Cortex	**Posterior Cingulate (23)**	3.70%	0.159	101
PM66-04	Cortex	PMC-paracentral gyrus	5.28%	0.09	287
PM66-04	Cortex	PMC-paracentral gyrus	5.49%	0.094	236
PM66-04	Cortex	**Retrosplenial (29/30- Superior CC**	n/a	n/a	n/a
PM66-04	Cortex	**Retrosplenial (29/30- Superior CC**	n/a	n/a	n/a
PM66-04	Cortex	**Retrosplenial (29/30- Inferior CC**	6.06%	0.13	92
PM66-04	Cortex	**Retrosplenial (29/30- Inferior CC**	6.52%	0.13	129
PM66-04	Cortex	**Superior Parietal**	5.94%	0.102	166
PM66-04	Cortex	**Superior Parietal**	5.23%	0.092	174
PM66-04	Cortex	**Occipitotemporal**	4.66%	0.111	149
PM66-04	Cortex	**Occipitotemporal**	3.55%	0.135	198
PM66-04	Cortex	Angular	3.01%	0.267	62
PM66-04	Cortex	Angular	2.10%	0.12	93
PM66-04	Cortex	V1	6.87%	0.126	98
PM66-04	Cortex	V1	11.87%	0.088	120
PM66-04	Cortex	Temporal Pole	12.36%	0.071	77
PM66-04	Cortex	Temporal Pole	5.87%	0.12	85
		Average	5.43%	0.1235	146.92
					287
					62

PM66-04	White Matter	**Prefrontal Cortex- Pole**	23.35%		0.03		65
PM66-04	White Matter	**Area 10**	25.24%		0.02		95
PM66-04	White Matter	Orbitofrontal Cortex	21.18%		0.03		67
PM66-04	White Matter	Rectus Gyrus	25.66%		0.02		96
PM66-04	White Matter	**Anterior Cingulate (24)**	16.27%		0.025		90
PM66-04	White Matter	**Anterior Cingulate (24)**	n/a	n/a		n/a	
PM66-04	White Matter	Primary Sensory cortex	21.22%		0.036		57
PM66-04	White Matter	Primary Sensory cortex	24.43%		0.024		65
PM66-04	White Matter	**Posterior Cingulate (23)**	14.06%		0.05		30
PM66-04	White Matter	**Posterior Cingulate (23)**	16.65%		0.035		50
PM66-04	White Matter	PMC-paracentral gyrus	24.41%		0.039		64
PM66-04	White Matter	PMC-paracentral gyrus	21.98%		0.026		90
PM66-04	White Matter	**Retrosplenial (29/30- Superior CC**	n/a	n/a		n/a	
PM66-04	White Matter	**Retrosplenial (29/30- Superior CC**	n/a	n/a		n/a	
PM66-04	White Matter	**Retrosplenial (29/30- Inferior CC**	30.70%		0.029		52
PM66-04	White Matter	**Retrosplenial (29/30- Inferior CC**	26.19%		0.038		45
PM66-04	White Matter	**Superior Parietal**	15.70%		0.034		51
PM66-04	White Matter	**Superior Parietal**	19.52%		0.03		53
PM66-04	White Matter	**Occipitotemporal**	17.59%		0.027		92
PM66-04	White Matter	**Occipitotemporal**	17.41%		0.049		26
PM66-04	White Matter	Angular	18.01%		0.084		18
PM66-04	White Matter	Angular	25.38%		0.027		70
PM66-04	White Matter	V1	36.31%		0.019		54
PM66-04	White Matter	V1	35.53%		0.015		66
PM66-04	White Matter	Temporal Pole	20.76%		0.074		13
PM66-04	White Matter	Temporal Pole	33.11%		0.039		42
		Average	23.07%		0.03		58.74
							96
							13

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM92-94	Cortex	**Prefrontal Cortex- Pole**	22.61%	0.048	73
PM92-94	Cortex	**Area 10**	22.16%	0.039	97
PM92-94	Cortex	Orbitofrontal Cortex	20.50%	0.067	58
PM92-94	Cortex	Rectus Gyrus	24.81%	0.03	132
PM92-94	Cortex	**Anterior Cingulate (24)**	20.85%	0.056	91
PM92-94	Cortex	**Anterior Cingulate (24)**	28.75%	0.043	53
PM92-94	Cortex	Primary Sensory cortex	22.08%	0.058	99
PM92-94	Cortex	Primary Sensory cortex	19.86%	0.04	97
PM92-94	Cortex	**Posterior Cingulate (23)**	19.74%	0.052	150
PM92-94	Cortex	**Posterior Cingulate (23)**	19.03%	0.058	153
PM92-94	Cortex	PMC-paracentral gyrus	16.56%	0.105	79
PM92-94	Cortex	PMC-paracentral gyrus	22.59%	0.048	116
PM92-94	Cortex	**Retrosplenial (29/30- Superior CC**	15.77%	0.052	124
PM92-94	Cortex	**Retrosplenial (29/30- Superior CC**	17.12%	0.077	89
PM92-94	Cortex	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a
PM92-94	Cortex	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a
PM92-94	Cortex	**Superior Parietal**	26.37%	0.048	53
PM92-94	Cortex	**Superior Parietal**	27.00%	0.045	60
PM92-94	Cortex	**Occipitotemporal**	12.29%	0.143	51
PM92-94	Cortex	**Occipitotemporal**	25.36%	0.05	99
PM92-94	Cortex	Angular	17.49%	0.096	67
PM92-94	Cortex	Angular	23.14%	0.054	50
PM92-94	Cortex	V1	18.17%	0.116	32
PM92-94	Cortex	V1	18.69%	0.084	41
PM92-94	Cortex	Temporal Pole	n/a	n/a	n/a
PM92-94	Cortex	Temporal Pole	n/a	n/a	n/a
		Average	20.95%	0.064045455	84.72727273
					153
					32

PM92-94	White Matter	**Prefrontal Cortex- Pole**	22.73%		0.042		34
PM92-94	White Matter	**Area 10**	20.33%		0.025		54
PM92-94	White Matter	Orbitofrontal Cortex	31.56%		0.038		34
PM92-94	White Matter	Rectus Gyrus	34.29%		0.037		40
PM92-94	White Matter	**Anterior Cingulate (24)**	40.44%		0.027		52
PM92-94	White Matter	**Anterior Cingulate (24)**	30.78%		0.065		35
PM92-94	White Matter	Primary Sensory cortex	n/a	n/a		n/a	
PM92-94	White Matter	Primary Sensory cortex	28.34%		0.028		49
PM92-94	White Matter	**Posterior Cingulate (23)**	43.95%		0.026		45
PM92-94	White Matter	**Posterior Cingulate (23)**	33.97%		0.028		68
PM92-94	White Matter	PMC-paracentral gyrus	36.61%		0.039		70
PM92-94	White Matter	PMC-paracentral gyrus	33.10%		0.06		59
PM92-94	White Matter	**Retrosplenial (29/30- Superior CC**	28.02%		0.035		78
PM92-94	White Matter	**Retrosplenial (29/30- Superior CC**	27.00%		0.038		47
PM92-94	White Matter	**Retrosplenial (29/30- Inferior CC**	n/a	n/a		n/a	
PM92-94	White Matter	**Retrosplenial (29/30- Inferior CC**	n/a	n/a		n/a	
PM92-94	White Matter	**Superior Parietal**	31.73%		0.032		25
PM92-94	White Matter	**Superior Parietal**	37.75%		0.058		36
PM92-94	White Matter	**Occipitotemporal**	23.05%		0.06		16
PM92-94	White Matter	**Occipitotemporal**	24.50%		0.033		57
PM92-94	White Matter	Angular	30.31%		0.061		15
PM92-94	White Matter	Angular	24.58%		0.032		68
PM92-94	White Matter	V1	34.57%		0.047		29
PM92-94	White Matter	V1	31.27%		0.028		26
PM92-94	White Matter	Temporal Pole	n/a	n/a		n/a	
PM92-94	White Matter	Temporal Pole	n/a	n/a		n/a	
		Average	30.90%		0.039952381		44.61904762
							78
							15

Identity	Area	Region	Vf	Coefficient Error	No. of Fields
PM64-07	Cortex	**Prefrontal Cortex- Pole**	26.41%	0.038	41
PM64-07	Cortex	**Area 10**	31.32%	0.045	46
PM64-07	Cortex	Orbitofrontal Cortex	18.76%	0.069	91
PM64-07	Cortex	Rectus Gyrus	21.89%	0.042	142
PM64-07	Cortex	**Anterior Cingulate (24)**	28.11%	0.02	95
PM64-07	Cortex	**Anterior Cingulate (24)**	24.14%	0.034	107
PM64-07	Cortex	Primary Sensory cortex	23.77%	0.045	72
PM64-07	Cortex	Primary Sensory cortex	35.73%	0.025	78
PM64-07	Cortex	**Posterior Cingulate (23)**	17.32%	0.059	132
PM64-07	Cortex	**Posterior Cingulate (23)**	19.92%	0.052	96
PM64-07	Cortex	PMC-paracentral gyrus	n/a	n/a	n/a
PM64-07	Cortex	PMC-paracentral gyrus	n/a	n/a	n/a
PM64-07	Cortex	**Retrosplenial (29/30- Superior CC**	24.15%	0.041	94
PM64-07	Cortex	**Retrosplenial (29/30- Superior CC**	30.56%	0.046	69
PM64-07	Cortex	**Retrosplenial (29/30- Inferior CC**	30.45%	0.041	53
PM64-07	Cortex	**Retrosplenial (29/30- Inferior CC**	25.33%	0.045	68
PM64-07	Cortex	**Superior Parietal**	26.90%	0.043	81
PM64-07	Cortex	**Superior Parietal**	27.50%	0.031	126
PM64-07	Cortex	**Occipitotemporal**	25.37%	0.062	53
PM64-07	Cortex	**Occipitotemporal**	31.41%	0.031	87
PM64-07	Cortex	Angular	28.48%	0.057	75
PM64-07	Cortex	Angular	32.92%	0.029	69
PM64-07	Cortex	V1	16.90%	0.067	47
PM64-07	Cortex	V1	31.08%	0.053	37
PM64-07	Cortex	Temporal Pole	28.45%	0.027	82
PM64-07	Cortex	Temporal Pole	37.22%	0.018	59
		Average	26.84%	0.0425	79.167
					142
					37

PM64-07	White Matter	**Prefrontal Cortex- Pole**	27.86%		0.029	56
PM64-07	White Matter	**Area 10**	33.27%		0.032	23
PM64-07	White Matter	Orbitofrontal Cortex	35.56%		0.035	23
PM64-07	White Matter	Rectus Gyrus	31.48%		0.019	58
PM64-07	White Matter	**Anterior Cingulate (24)**	34.13%		0.03	46
PM64-07	White Matter	**Anterior Cingulate (24)**	31.03%		0.035	33
PM64-07	White Matter	Primary Sensory cortex	24.42%		0.022	85
PM64-07	White Matter	Primary Sensory cortex	39.58%		0.037	28
PM64-07	White Matter	**Posterior Cingulate (23)**	32.99%		0.013	84
PM64-07	White Matter	**Posterior Cingulate (23)**	33.58%		0.031	40
PM64-07	White Matter	PMC-paracentral gyrus	n/a	n/a	n/a	
PM64-07	White Matter	PMC-paracentral gyrus	n/a	n/a	n/a	
PM64-07	White Matter	**Retrosplenial (29/30- Superior CC**	47.41%		0.017	73
PM64-07	White Matter	**Retrosplenial (29/30- Superior CC**	31.07%		0.028	38
PM64-07	White Matter	**Retrosplenial (29/30- Inferior CC**	53.19%		0.024	31
PM64-07	White Matter	**Retrosplenial (29/30- Inferior CC**	38.64%		0.025	29
PM64-07	White Matter	**Superior Parietal**	34.17%		0.017	55
PM64-07	White Matter	**Superior Parietal**	34.62%		0.021	27
PM64-07	White Matter	**Occipitotemporal**	44.37%		0.056	18
PM64-07	White Matter	**Occipitotemporal**	68.20%		0.011	28
PM64-07	White Matter	Angular	37.69%		0.02	73
PM64-07	White Matter	Angular	39.60%		0.059	14
PM64-07	White Matter	V1	36.79%		0.03	12
PM64-07	White Matter	V1	36.60%		0.06	22
PM64-07	White Matter	Temporal Pole	31.34%		0.012	36
PM64-07	White Matter	Temporal Pole	39.03%		0.019	18
		Average	37.36%		0.028	39.58
						85
						12

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM51-06	Cortex	**Prefrontal Cortex- Pole**	21.39%	0.08	66
PM51-06	Cortex	**Area 10**	20.53%	0.06	95
PM51-06	Cortex	Orbitofrontal Cortex	21.61%	0.066	67
PM51-06	Cortex	Rectus Gyrus	19.10%	0.053	165
PM51-06	Cortex	**Anterior Cingulate (24)**	23.26%	0.055	125
PM51-06	Cortex	**Anterior Cingulate (24)**	22.33%	0.052	119
PM51-06	Cortex	Primary Sensory cortex	15.90%	0.05	152
PM51-06	Cortex	Primary Sensory cortex	11.78%	0.082	104
PM51-06	Cortex	**Posterior Cingulate (23)**	11.12%	0.066	160
PM51-06	Cortex	**Posterior Cingulate (23)**	17.64%	0.054	139
PM51-06	Cortex	PMC-paracentral gyrus	18.05%	0.05	72
PM51-06	Cortex	PMC-paracentral gyrus	13.72%	0.097	77
PM51-06	Cortex	**Retrosplenial (29/30- Superior CC**	17.88%	0.046	136
PM51-06	Cortex	**Retrosplenial (29/30- Superior CC**	18.10%	0.136	49
PM51-06	Cortex	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a
PM51-06	Cortex	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a
PM51-06	Cortex	**Superior Parietal**	25.57%	0.033	109
PM51-06	Cortex	**Superior Parietal**	18.04%	0.099	74
PM51-06	Cortex	**Occipitotemporal**	14.88%	0.05	169
PM51-06	Cortex	**Occipitotemporal**	11.08%	0.058	134
PM51-06	Cortex	Angular	9.21%	0.087	110
PM51-06	Cortex	Angular	16.73%	0.058	118
PM51-06	Cortex	V1	10.94%	0.109	75
PM51-06	Cortex	V1	14.23%	0.104	87
PM51-06	Cortex	Temporal Pole	n/a	n/a	n/a
PM51-06	Cortex	Temporal Pole	n/a	n/a	n/a
		Average	16.96%	0.070227273	109.1818182
					169
					49

PM51-06	White Matter	**Prefrontal Cortex- Pole**		30.95%	0.018	44
PM51-06	White Matter	**Area 10**		28.46%	0.019	60
PM51-06	White Matter	Orbitofrontal Cortex		53.63%	0.09	15
PM51-06	White Matter	Rectus Gyrus		26.35%	0.024	32
PM51-06	White Matter	**Anterior Cingulate (24)**		19.28%	0.04	31
PM51-06	White Matter	**Anterior Cingulate (24)**		23.22%	0.024	44
PM51-06	White Matter	Primary Sensory cortex		18.98%	0.043	28
PM51-06	White Matter	Primary Sensory cortex		24.44%	0.046	26
PM51-06	White Matter	**Posterior Cingulate (23)**		28.06%	0.036	32
PM51-06	White Matter	**Posterior Cingulate (23)**		17.53%	0.049	21
PM51-06	White Matter	PMC-paracentral gyrus		29.61%	0.026	24
PM51-06	White Matter	PMC-paracentral gyrus		26.68%	0.019	53
PM51-06	White Matter	**Retrosplenial (29/30- Superior CC**		21.96%	0.034	35
PM51-06	White Matter	**Retrosplenial (29/30- Superior CC**		22.80%	0.025	40
PM51-06	White Matter	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a	
PM51-06	White Matter	**Retrosplenial (29/30- Inferior CC**	n/a	n/a	n/a	
PM51-06	White Matter	**Superior Parietal**		37.96%	0.033	23
PM51-06	White Matter	**Superior Parietal**		21.70%	0.049	26
PM51-06	White Matter	**Occipitotemporal**		21.86%	0.061	18
PM51-06	White Matter	**Occipitotemporal**		29.58%	0.052	21
PM51-06	White Matter	Angular		19.84%	0.053	32
PM51-06	White Matter	Angular		29.23%	0.036	31
PM51-06	White Matter	V1		48.20%	0.028	31
PM51-06	White Matter	V1		33.52%	0.025	32
PM51-06	White Matter	Temporal Pole	n/a	n/a	n/a	
PM51-06	White Matter	Temporal Pole	n/a	n/a	n/a	
		Average		27.90%	0.037727273	31.77272727
						60
						15

Identity	Area	Region	Vf	Coefficient Error	No.Of Fields
PM45-98	Cortex	**Prefrontal Cortex- Pole**	0.98%	0.119	62
PM45-98	Cortex	**Area 10**	n/a	n/a	n/a
PM45-98	Cortex	Orbitofrontal Cortex	6.48%	0.159	98
PM45-98	Cortex	Rectus Gyrus	3.67%	0.12	95
PM45-98	Cortex	**Anterior Cingulate (24)**	5.47%	0.072	103
PM45-98	Cortex	**Anterior Cingulate (24)**	6.37%	0.06	63
PM45-98	Cortex	Primary Sensory cortex	5.92%	0.118	175
PM45-98	Cortex	Primary Sensory cortex	5.37%	0.116	155
PM45-98	Cortex	**Posterior Cingulate (23)**	4.37%	0.044	113
PM45-98	Cortex	**Posterior Cingulate (23)**	7.31%	0.156	97
PM45-98	Cortex	PMC-paracentral gyrus	4.24%	0.084	148
PM45-98	Cortex	PMC-paracentral gyrus	6.32%	0.054	134
PM45-98	Cortex	**Retrosplenial (29/30- Superior CC**	3.87%	0.054	171
PM45-98	Cortex	**Retrosplenial (29/30- Superior CC**	4.03%	0.041	126
PM45-98	Cortex	**Retrosplenial (29/30- Inferior CC**	11.04%	0.14	95
PM45-98	Cortex	**Retrosplenial (29/30- Inferior CC**	5.62%	0.15	94
PM45-98	Cortex	**Superior Parietal**	2.70%	0.075	70
PM45-98	Cortex	**Superior Parietal**	3.01%	0.107	89
PM45-98	Cortex	**Occipitotemporal**	2.15%	0.042	154
PM45-98	Cortex	**Occipitotemporal**	4.36%	0.1	150
PM45-98	Cortex	Angular	n/a	n/a	n/a
PM45-98	Cortex	Angular	n/a	n/a	n/a
PM45-98	Cortex	V1	2.72%	0.079	81
PM45-98	Cortex	V1	3.30%	0.088	91
PM45-98	Cortex	Temporal Pole	n/a	n/a	n/a
PM45-98	Cortex	Temporal Pole	n/a	n/a	n/a
		Average	4.73%	0.094	112.57
					175
					62

PM45-98	White Matter	**Prefrontal Cortex- Pole**	20.70%		0.026		60
PM45-98	White Matter	**Area 10**	n/a	n/a		n/a	
PM45-98	White Matter	Orbitofrontal Cortex	7.86%		0.125		25
PM45-98	White Matter	Rectus Gyrus	6.64%		0.104		32
PM45-98	White Matter	**Anterior Cingulate (24)**	8.72%		0.071		36
PM45-98	White Matter	**Anterior Cingulate (24)**	13.29%		0.043		35
PM45-98	White Matter	Primary Sensory cortex	6.57%		0.042		54
PM45-98	White Matter	Primary Sensory cortex	7.39%		0.077		34
PM45-98	White Matter	**Posterior Cingulate (23)**	13.83%		0.026		68
PM45-98	White Matter	**Posterior Cingulate (23)**	12.35%		0.03		63
PM45-98	White Matter	PMC-paracentral gyrus	20.76%		0.027		55
PM45-98	White Matter	PMC-paracentral gyrus	6.59%		0.1		25
PM45-98	White Matter	**Retrosplenial (29/30- Superior CC**	4.89%		0.07		49
PM45-98	White Matter	**Retrosplenial (29/30- Superior CC**	6.96%		0.079		41
PM45-98	White Matter	**Retrosplenial (29/30- Inferior CC**	38.03%		0.038		45
PM45-98	White Matter	**Retrosplenial (29/30- Inferior CC**	16.23%		0.066		49
PM45-98	White Matter	**Superior Parietal**	6.30%		0.07		34
PM45-98	White Matter	**Superior Parietal**	5.39%		0.066		35
PM45-98	White Matter	**Occipitotemporal**	10.00%		0.109		6
PM45-98	White Matter	**Occipitotemporal**	14.11%		0.027		60
PM45-98	White Matter	Angular	n/a	n/a		n/a	
PM45-98	White Matter	Angular	n/a	n/a		n/a	
PM45-98	White Matter	V1	19.26%		0.04		42
PM45-98	White Matter	V1	13.84%		0.044		50
PM45-98	White Matter	Temporal Pole	n/a	n/a		n/a	
PM45-98	White Matter	Temporal Pole	n/a	n/a		n/a	
		Average	12.37%		0.061		42.76
							68
							6

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM12-98	Cortex	**Prefrontal Cortex- Pole**	7.56%	0.105	94
PM12-98	Cortex	**Area 10**	9.57%	0.073	101
PM12-98	Cortex	Orbitofrontal Cortex	9.35%	0.089	79
PM12-98	Cortex	Rectus Gyrus	7.38%	0.073	161
PM12-98	Cortex	**Anterior Cingulate (24)**	7.91%	0.077	77
PM12-98	Cortex	**Anterior Cingulate (24)**	9.51%	0.082	83
PM12-98	Cortex	Primary Sensory cortex	8.48%	0.045	256
PM12-98	Cortex	Primary Sensory cortex	5.93%	0.064	173
PM12-98	Cortex	**Posterior Cingulate (23)**	9.50%	0.093	98
PM12-98	Cortex	**Posterior Cingulate (23)**	7.75%	0.079	179
PM12-98	Cortex	PMC-paracentral gyrus	9.14%	0.053	172
PM12-98	Cortex	PMC-paracentral gyrus	10.22%	0.034	379
PM12-98	Cortex	**Retrosplenial (29/30- Superior CC**	9.06%	0.069	141
PM12-98	Cortex	**Retrosplenial (29/30- Superior CC**	5.38%	0.103	70
PM12-98	Cortex	**Retrosplenial (29/30- Inferior CC**	15.28%	0.066	73
PM12-98	Cortex	**Retrosplenial (29/30- Inferior CC**	15.44%	0.095	75
PM12-98	Cortex	**Superior Parietal**	11.82%	0.061	108
PM12-98	Cortex	**Superior Parietal**	10.50%	0.072	75
PM12-98	Cortex	**Occipitotemporal**	7.37%	0.109	64
PM12-98	Cortex	**Occipitotemporal**	8.21%	0.046	200
PM12-98	Cortex	Angular	8.05%	0.1	100
PM12-98	Cortex	Angular	8.27%	0.07	143
PM12-98	Cortex	V1	4.63%	0.079	104
PM12-98	Cortex	V1	5.01%	0.053	70
PM12-98	Cortex	Temporal Pole	n/a	n/a	n/a
PM12-98	Cortex	Temporal Pole	n/a	n/a	n/a
		Average	8.81%	0.074583333	128.125
					379
					64

PM12-98	White Matter	**Prefrontal Cortex- Pole**	11.78%		0.034	53
PM12-98	White Matter	**Area 10**	10.94%		0.029	67
PM12-98	White Matter	Orbitofrontal Cortex	17.76%		0.041	60
PM12-98	White Matter	Rectus Gyrus	14.00%		0.032	84
PM12-98	White Matter	**Anterior Cingulate (24)**	13.12%		0.041	36
PM12-98	White Matter	**Anterior Cingulate (24)**	6.69%		0.109	28
PM12-98	White Matter	Primary Sensory cortex	9.99%		0.05	39
PM12-98	White Matter	Primary Sensory cortex	8.33%		0.072	31
PM12-98	White Matter	**Posterior Cingulate (23)**	7.89%		0.048	68
PM12-98	White Matter	**Posterior Cingulate (23)**	11.16%		0.032	78
PM12-98	White Matter	PMC-paracentral gyrus	9.72%		0.044	45
PM12-98	White Matter	PMC-paracentral gyrus	9.27%		0.029	102
PM12-98	White Matter	**Retrosplenial (29/30- Superior CC**	7.91%		0.064	73
PM12-98	White Matter	**Retrosplenial (29/30- Superior CC**	6.54%		0.079	45
PM12-98	White Matter	**Retrosplenial (29/30- Inferior CC**	16.32%		0.065	14
PM12-98	White Matter	**Retrosplenial (29/30- Inferior CC**	18.73%		0.056	16
PM12-98	White Matter	**Superior Parietal**	10.65%		0.072	45
PM12-98	White Matter	**Superior Parietal**	13.06%		0.058	24
PM12-98	White Matter	**Occipitotemporal**	12.97%		0.069	13
PM12-98	White Matter	**Occipitotemporal**	11.04%		0.043	32
PM12-98	White Matter	Angular	6.74%		0.108	24
PM12-98	White Matter	Angular	12.12%		0.04	38
PM12-98	White Matter	V1	24.41%		0.054	33
PM12-98	White Matter	V1	10.44%		0.048	33
PM12-98	White Matter	Temporal Pole	n/a	n/a	n/a	
PM12-98	White Matter	Temporal Pole	n/a	n/a	n/a	
		Average	11.73%		0.054875	45.04166667
						102
						13

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM44-98	Cortex	**Prefrontal Cortex- Pole**	7.12%	0.117	74
PM44-98	Cortex	**Area 10**	6.53%	0.166	79
PM44-98	Cortex	Orbitofrontal Cortex	2.39%	0.136	72
PM44-98	Cortex	Rectus Gyrus	2.49%	0.297	38
PM44-98	Cortex	**Anterior Cingulate (24)**	3.49%	0.1	128
PM44-98	Cortex	**Anterior Cingulate (24)**	24.28%	0.058	82
PM44-98	Cortex	Primary Sensory cortex	5.81%	0.111	177
PM44-98	Cortex	Primary Sensory cortex	5.80%	0.154	126
PM44-98	Cortex	**Posterior Cingulate (23)**	3.59%	0.199	93
PM44-98	Cortex	**Posterior Cingulate (23)**	2.77%	0.196	76
PM44-98	Cortex	PMC-paracentral gyrus	3.84%	0.175	102
PM44-98	Cortex	PMC-paracentral gyrus	1.76%	0.118	88
PM44-98	Cortex	**Retrosplenial (29/30- Superior CC**	4.34%	0.173	91
PM44-98	Cortex	**Retrosplenial (29/30- Superior CC**	2.50%	0.222	82
PM44-98	Cortex	**Retrosplenial (29/30- Inferior CC**	2.74%	0.27	39
PM44-98	Cortex	**Retrosplenial (29/30- Inferior CC**	2.15%	0.239	94
PM44-98	Cortex	**Superior Parietal**	4.31%	0.217	64
PM44-98	Cortex	**Superior Parietal**	2.92%	0.21	51
PM44-98	Cortex	**Occipitotemporal**	5.83%	0.207	47
PM44-98	Cortex	**Occipitotemporal**	4.63%	0.198	71
PM44-98	Cortex	Angular	5.18%	0.16	84
PM44-98	Cortex	Angular	4.24%	0.178	71
PM44-98	Cortex	V1	5.51%	0.187	71
PM44-98	Cortex	V1	4.44%	0.178	52
PM44-98	Cortex	Temporal Pole	3.22%	0.195	112
PM44-98	Cortex	Temporal Pole	2.46%	0.17	67
		Average	4.78%	0.178115385	81.96153846
					177
					38

PM44-98	White Matter	**Prefrontal Cortex- Pole**	12.87%	0.028	89
PM44-98	White Matter	**Area 10**	13.59%	0.021	126
PM44-98	White Matter	Orbitofrontal Cortex	12.36%	0.06	42
PM44-98	White Matter	Rectus Gyrus	18.71%	0.05	76
PM44-98	White Matter	**Anterior Cingulate (24)**	10.66%	0.048	45
PM44-98	White Matter	**Anterior Cingulate (24)**	18.03%	0.056	31
PM44-98	White Matter	Primary Sensory cortex	8.72%	0.048	84
PM44-98	White Matter	Primary Sensory cortex	6.20%	0.077	88
PM44-98	White Matter	**Posterior Cingulate (23)**	9.87%	0.047	114
PM44-98	White Matter	**Posterior Cingulate (23)**	5.83%	0.065	49
PM44-98	White Matter	PMC-paracentral gyrus	12.94%	0.064	54
PM44-98	White Matter	PMC-paracentral gyrus	12.84%	0.054	58
PM44-98	White Matter	**Retrosplenial (29/30- Superior CC**	8.62%	0.036	99
PM44-98	White Matter	**Retrosplenial (29/30- Superior CC**	8.78%	0.056	80
PM44-98	White Matter	**Retrosplenial (29/30- Inferior CC**	16.77%	0.09	35
PM44-98	White Matter	**Retrosplenial (29/30- Inferior CC**	20.85%	0.058	64
PM44-98	White Matter	**Superior Parietal**	12.38%	0.04	73
PM44-98	White Matter	**Superior Parietal**	12.12%	0.053	52
PM44-98	White Matter	**Occipitotemporal**	10.74%	0.06	28
PM44-98	White Matter	**Occipitotemporal**	9.53%	0.043	33
PM44-98	White Matter	Angular	12.25%	0.065	51
PM44-98	White Matter	Angular	10.79%	0.079	26
PM44-98	White Matter	V1	23.73%	0.032	41
PM44-98	White Matter	V1	31.00%	0.025	46
PM44-98	White Matter	Temporal Pole	8.74%	0.0114	29
PM44-98	White Matter	Temporal Pole	16.31%	0.041	55
			13.28%	0.050284615	60.30769231
					126
					26

Identity	Area	Region	Vf	Coefficient Error	No. Of Fields
PM42-97	Cortex	**Prefrontal Cortex- Pole**	2.01%	0.281	81
PM42-97	Cortex	**Area 10**	0.44%	0.126	102
PM42-97	Cortex	Orbitofrontal Cortex	0.30%	0.09	136
PM42-97	Cortex	Rectus Gyrus	0.20%	0.08	129
PM42-97	Cortex	**Anterior Cingulate (24)**	0.78%	0.179	99
PM42-97	Cortex	**Anterior Cingulate (24)**	0.55%	0.178	185
PM42-97	Cortex	Primary Sensory cortex	1.59%	0.279	92
PM42-97	Cortex	Primary Sensory cortex	0.43%	0.116	88
PM42-97	Cortex	**Posterior Cingulate (23)**	0.04%	0.143	131
PM42-97	Cortex	**Posterior Cingulate (23)**	0.57%	0.149	123
PM42-97	Cortex	PMC-paracentral gyrus	1.84%	0.153	361
PM42-97	Cortex	PMC-paracentral gyrus	0.73%	0.268	175
PM42-97	Cortex	**Retrosplenial (29/30- Superior CC**	0.46%	0.188	113
PM42-97	Cortex	**Retrosplenial (29/30- Superior CC**	0.60%	0.287	79
PM42-97	Cortex	**Retrosplenial (29/30- Inferior CC**	0.51%	0.365	93
PM42-97	Cortex	**Retrosplenial (29/30- Inferior CC**	0.84%	0.252	103
PM42-97	Cortex	**Superior Parietal**	1.63%	0.224	136
PM42-97	Cortex	**Superior Parietal**	0.24%	0.158	77
PM42-97	Cortex	**Occipitotemporal**	0.43%	0.205	95
PM42-97	Cortex	**Occipitotemporal**	0.40%	0.251	104
PM42-97	Cortex	Angular	0.76%	0.276	141
PM42-97	Cortex	Angular	1.08%	0.247	111
PM42-97	Cortex	V1	0.40%	0.23	85
PM42-97	Cortex	V1	0.50%	0.112	76
PM42-97	Cortex	Temporal Pole	n/a	n/a	n/a
PM42-97	Cortex	Temporal Pole	n/a	n/a	n/a
		Average	0.72%	0.201541667	121.4583333
					361
					76

PM42-97	White Matter	**Prefrontal Cortex- Pole**	1.77%		0.043	99
PM42-97	White Matter	**Area 10**	0.91%		0.047	131
PM42-97	White Matter	Orbitofrontal Cortex	0.89%		0.05	120
PM42-97	White Matter	Rectus Gyrus	1.24%		0.048	105
PM42-97	White Matter	**Anterior Cingulate (24)**	2.09%		0.075	77
PM42-97	White Matter	**Anterior Cingulate (24)**	2.16%		0.107	70
PM42-97	White Matter	Primary Sensory cortex	0.75%		0.12	28
PM42-97	White Matter	Primary Sensory cortex	0.94%		0.096	114
PM42-97	White Matter	**Posterior Cingulate (23)**	1.52%		0.075	45
PM42-97	White Matter	**Posterior Cingulate (23)**	0.85%		0.065	48
PM42-97	White Matter	PMC-paracentral gyrus	0.93%		0.12	105
PM42-97	White Matter	PMC-paracentral gyrus	0.68%		0.074	92
PM42-97	White Matter	**Retrosplenial (29/30- Superior CC**	0.76%		0.088	61
PM42-97	White Matter	**Retrosplenial (29/30- Superior CC**	0.85%		0.101	45
PM42-97	White Matter	**Retrosplenial (29/30- Inferior CC**	1.34%		0.098	71
PM42-97	White Matter	**Retrosplenial (29/30- Inferior CC**	2.41%		0.115	29
PM42-97	White Matter	**Superior Parietal**	1.09%		0.082	48
PM42-97	White Matter	**Superior Parietal**	0.48%		0.114	96
PM42-97	White Matter	**Occipitotemporal**	1.12%		0.152	34
PM42-97	White Matter	**Occipitotemporal**	1.00%		0.2	50
PM42-97	White Matter	Angular	0.68%		0.076	81
PM42-97	White Matter	Angular	1.10%		0.159	44
PM42-97	White Matter	V1	1.84%		0.084	71
PM42-97	White Matter	V1	2.42%		0.05	79
PM42-97	White Matter	Temporal Pole	n/a	n/a	n/a	
PM42-97	White Matter	Temporal Pole	n/a	n/a	n/a	
		Average	1.24%		0.093291667	72.625
						131
						28